ANALYSIS OF THE RESPONSES OF YARD LONG BEAN TO WATERLOGGING

EIJI NAWATA

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Contents

Chapter 1. Introduction

It is well recognized that waterlogging of the soil causes a reduction of growth and yield in various crops (Cannel et al., 1979, Forsythe et al., 1979; Klrizek, 1982; Heinrichs, 1970; Jackson, 1979; Kuo and Chen, 1980; Minchin et al., 1978; Rowe and Beardsell, 1973; Sugimoto et al., 1988; Wien et al., 1979). Once soil is waterlogged, the air space is displaced with water, and the O_2 remaining in the soil is quickly depleted by the respiration of plant roots and soil microorganisms with slow diffusion of atmospheric O_2. Thus plant roots are suddenly exposed to anaerobic conditions and growth and development are inhibited (Kawase,1981). At the same time, the anaerobic conditions affect the activities of soil microorganisms and change the biological environment of plant roots drastically. The adverse effects of waterlogging on plant growth result directly from restricted root growth caused by the suppression of aerobic respiration, and indirectly from the interaction of plant root and soil microorganisms (Glinski and Stepniewski, 1985; Nawata and Shigenaga, 1988).

In tropical Asia, heavy rainfall in the rainy season frequently induces short-term flooding in crop fields. This phenomenon is accelerated by poor drainage system, uneven local topography and high ground water level. Even in the dry season, some cultural practices to maintain available water sometimes cause transient soil submersion in crop fields. Under these conditions, crops cultivated are damaged to various degrees, resulting in poor growth and low yield.

Yard long bean is widely cultivated for young pod consumption in East, Southeast and South Asia. Especially in tropical Asia, this crop is one of the most important vegetables cultivated all the year round. For the above-mentioned reasons, yard long bean plants are sometimes subjected to the detrimental effect of waterlogging. Additionally, this crop is generally considered to be susceptible to waterlogging damages. This problem is one of the major factors which restrict the productivity of this crop in tropical Asia.

Ritchie (1980) stated that the development of an optimum production system which has constraints from plant water deficits is consistent of two equally important parts, i.e. to design through breeding and selection plants that are best suited to the environment, and to manage plants to suit the environment. This statement is directly applicable for the methods to lighten waterlogging damages. For both the breeding of tolerant plants and the improvement of cultural practices for better plant management, it is indispensable to obtain fundamental information about the response of yard long bean plants to waterlogging.

Although some information is available in other leguminous crops (Minchin and Summerfield, 1976; Wadman-van and Van Andel, 1985; Wien et al.,1979), detailed studies on the response of yard long bean plants to waterlogging have not been conducted so far.

This study was carried out to elucidate various factors which affect the responses of yard long bean to waterlogging, to discuss problems relating to selection and breeding for waterlogging tolerance and cultural practices for preventing flooding damages and finally to find out the effective methods to improve the productivity of this crop in tropical Asia, under the environment where waterlogging damages frequently occur.

Chapter 2. Various factors affecting the responses of yard long bean to waterlogging

Section 1. Effects of the stage of growth and Rhizobium inoculation

Introduction

Many factors are known to affect the damages caused by soil flooding. Those include duration of waterlogging, the stage of the growth, the species and/or varieties and environmental factors such as temperature and soil characteristics (Krizek, 1982). In this chapter, several experiments were carried out to clarify how some of those factors influence the response of yard long bean to waterlogging.

The response of crop plants to waterlogging depends on the stage of growth when it is experienced (Letey et al., 1961; Krizek, 1982). In many crops, just prior to flowering or during early flower development is known to be the most sensitive stages to waterlogging (Cannell et. al., 1979; Krizek, 1982). However, Minchin et al. (1978) demonstrated that in cowpeas the biggest yield reduction was observed in plants subjected to short-term waterlogging at the vegetative stage. The similar result was obtained by Sugimoto et al. (1988) in soybean.

Soil anaerobiosis induced by soil flooding also affects the activities of nitrogen fixing Rhizobia adversely. In solution culture, nodulation of garden pea roots were severely retarded by low oxygen concentration of nutrient solution (Minchin and Pate, 1975). Minchin and Summerfield (1976) observed that the yield reduction of cowpea by short-term waterlogging was more in inoculated plants than that in non-inoculated, inorganic nitrogen supplied plants. They considered that this phenomenon was caused by nutrient deficiency during the period of post-stress recovery of the activities of Rhizobia.

This experiment was designed mainly to clarify the effect of short-term waterlogging at the different stages of growth and Rhizobia inoculation on the growth and yield of yard long bean plant.

Another purpose of this experiment was to elucidate the interrelationship between the effects of waterlogging on the seed yield and those on the pod yield. If there is a positive correlation between them, only seed yield should be determined in this kind of study because of simplicity of measurement.

In this experiment, soils were sterilized by fumigation before use to minimize the interaction between plants and soil microorganisms and evaluate the direct influence of soil anaerobiosis on plants.

Materials and Methods

Yard long bean cv. "TKC-83", a Thai local variety and relatively photo-insensitive for the flower response, were sown in vermiculite on May 6, 1986 after sterilization with 1 % commercial fungicide, "Benlate" ("Dupont" Co.Ltd.)

for 10 min. On May 20, the seedlings were transplanted in 24 cm diameter clay pots containing soil mixture of sand and commercial compost "Rock compo" ("Marutane Seed" Co.Ltd.) at the ratio of 2:1. Plants were grown in a plastic greenhouse without control of temperature. Fluctuation of ambient temperature during this experiment is shown in Fig.1.

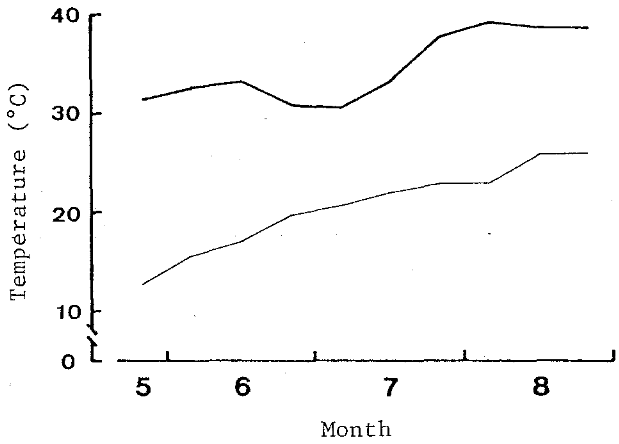

Fig. 1. The fluctuation of ambient maximum (—) and minimum (-) temperature during the experiment.

Experiment for yield determination was conducted as a completely randomized design with 8 replications in a factorial arrangement of 3 treatments concerning waterlogging (including non-waterlogged control) and 2 nitrogen application methods. Half of the replications was for measuring seed yield and the other half was for young pod yield. Plants were spaced 30 cm in-row and 70 cm between-row.

Waterlogging treatment was applied by placing the 24 cm diameter pots into basally sealed 30 cm diameter pots and filling the outer containers with tap water. The water surface was kept at 2 cm above the soil level. Appropriate number of plants were subjected to waterlogging treatment for 4 days at the vegetative stage or flowering stage. The treatment at the vegetative stage began at 27 days after sowing when 2-3 trifoliate leaves were expanded. The treatment at the flowering stage was started at 57 days after sowing when the first flowers just opened. Before the beginning of the treatment remaining nutrients were flushed through each pot with 3-4 l tap water to adjust the nutrient condition in the potted soil. At the end of the waterlogging periods, each treated plant was returned to a free-draining state and allowed to stand for up to 2-3 days before rewatering with appropriate nutrient solution.

Three days after transplanting half of the plants was inoculated with Rhizobium sp. (cowpea group), race "NIASE-3063". Non-inoculated plants received standard "ENSHI" nutrient solution (Yamazaki, 1982) containing 16 me/l N, whereas inoculated plants were irrigated by modified "ENSHI" nutrient solution containing 1 me/l N, daily except during waterlogging treatment. When waterlogging treatment was applied, tap water was supplied to non-waterlogged plants. In modified "ENSHI" nutrient solution NO_3^- was replaced with Cl^-.

In plants for seed yield determination, each pod was harvested when it was physiologically matured. Harvested pods were oven-dried at 35 °C for 2 days and weighed. Harvesting was continued until each plant was dried up. In plants for young pod harvesting, each pod was harvested at about 9 days after its flowering and weighed immediately. When young pods of yard long bean are continuously harvested, a resting stage for certain period in terms of flowering and podding comes after highly producing period. Shortly after this stage flowering and podding resume. Usually this cycle is repeated until plant senescence. In this experiment, harvesting of young pods was terminated when the second cycle was over in each plant. In both seed and young pod harvesting, the number of days to first flower, flower number, and various characteristics relating to the yield were also determined.

Plants for growth analysis of vegetative organs were separately grown and arranged at the same plant spacing in the same plastic greenhouse. They received the same treatments as those for yield determination, and 4 plants in each treatment plot were sampled at 27 (just before the beginning of the waterlogging treatment at the vegetative stage), 31 (just after the removal of waterlogging treatment at the vegetative stage), 41, 51 and 62 (just after the removal of waterlogging treatment at the flowering stage) days after sowing. All sampled plants were separated into their various components and fresh and dry weights were recorded.

Results

The growth rate of non-inoculated, inorganic nitrogen supplied plants were much higher than inoculated ones (Fig.2 and Fig.3). In plants subjected to short-term

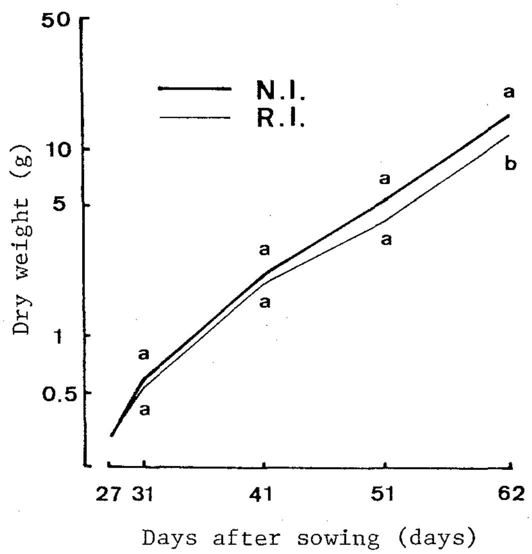

Fig. 2 The effect of Rhizobium inoculation on the changes in dry weight of above-ground parts. N.I.; non-inoculated plants, R.I.; Rhizobium inoculated plants. Different letters at each measurement time indicate significant difference at p=0.05.

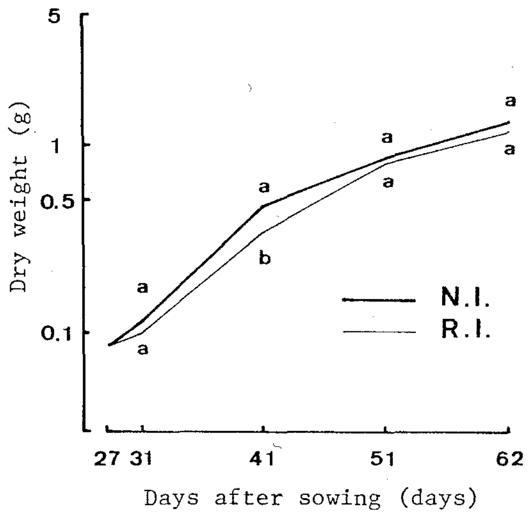

Fig. 3 The effect of Rhizobium inoculation on the changes in dry weight of roots. Abbreviations for treatments are the same as those in Fig. 2. Different letters at each measurement time indicate significant difference at p=0.05.

waterlogging, stem hypertrophy and the formation of white and spongy tissue were observed irrespective of the stage of the treatment. Such tissue was dried after the removal of the treatment. Fig.4 and Fig.5 show the effects of short-term waterlogging on growth. In the above-ground parts, the growth had been retarded by waterlogging treatments, whereas in roots rapid recovery could be seen soon after the treatment. After recovering period, root growth was also reduced. Fresh and dry weights of the above-ground parts were not affected just after the beginning of the waterlogging treatment, but those of roots were significantly reduced by the treatment. The significant interaction between the effect of waterlogging and inoculation was not observed in each measurement time in both plant components.

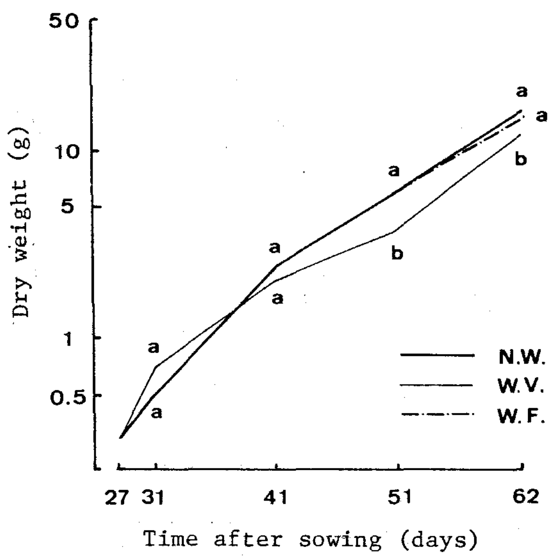

Fig. 4 The effect of short-term waterlogging on the changes in above-ground parts. N.W.; non-waterlogged, W.V.; subjected to waterlogging at the vegetative stage, W.F.; subjected to waterlogging at the flowering stage. Different letters at each measurement time indicate significant difference at p=0.05.

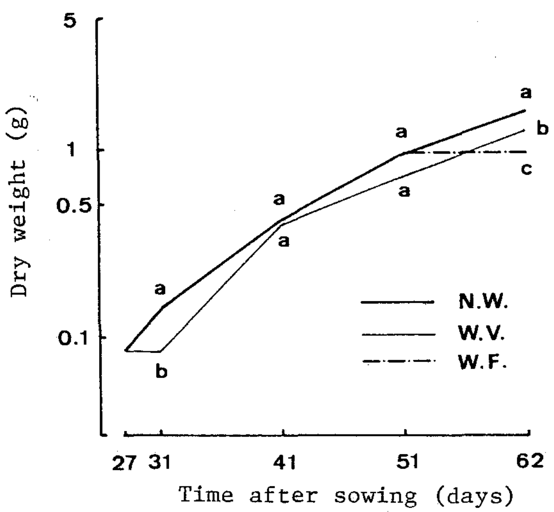

Fig. 5 The effect of short-term waterlogging on the changes in roots. Abbreviations for treatments are the same as those in Fig. 4. Different letters at each measurement time indicate significant difference at p=0.05.

The number of days to first flower was not affected by any treatments (Table 1). The significant interaction between the effect of Rhizobium inoculation and waterlogging was not found.

Table 1 The effect of Rhizobium inoculation and short-term waterlogging on number of days to first flower.

Treatment[1)]	Number of days to first flower (days)
N.I.	57.2 A[2)]
R.I.	59.6 A
N.W.	58.2 a[3)]
W.V.	58.7 a
W.F.	58.3 a

1) Abbreviations for treatments are the same as those in Fig. 4.
2),3) Different letters indicate significant difference at P=0.05.

Table 2 The effect of Rhizobium inoculation and short-term waterlogging on flower number in seed harvesting of yard long bean.

Treatment[1]	Flower number	
	main stem	lateral shoots
N.I.	23.2 A[2]	9.6 A
R.I.	18.3 B	4.8 B
N.W.	20.5 a[3]	11.4 a
W.V.	23.3 a	2.0 b
W.F.	18.4 a	8.1 a

1) Abbreviations for treatments are the same as those in Fig. 4.
2),3) Different letters indicate significant difference at P=0.05.

The number of flowers on both main stem and lateral shoots were significantly higher in non-inoculated plants than in inoculated ones as shown in Table 2. The number of flowers on main stem was not affected by waterlogging treatments, but those on lateral shoots were reduced significantly by waterlogging at the vegetative stage. The significant interaction between the effect of waterlogging and Rhizobium inoculation was not found in both of the parameters.

The seed yield was significantly higher in non-inoculated plants than in Rhizobium inoculated plants (Fig.6). The seed yield in plants subjected to waterlogging at the vegetative stage was significantly lower than those subjected to the other treatments. The effect of waterlogging at the flowering stage was not significant. The interaction between Rhizobium inoculation and waterlogging was not observed.

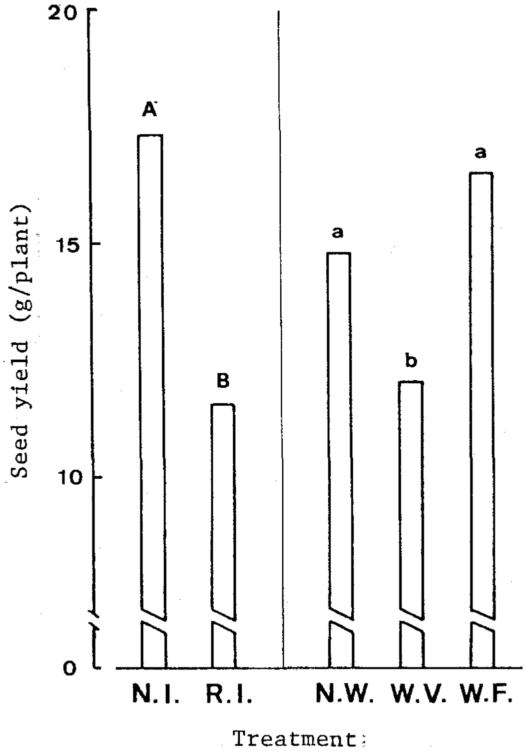

Fig. 6 The effect of Rhizobium inoculation and short-term waterlogging on seed yield. Abbreviations for treatments are the same as those in the previous figures. Columns covered by the same letter do not differ at the 5 % level.

The effects of Rhizobium inoculation and short-term waterlogging on various characteristics relating to the seed yield are shown in Table 3. Significant difference between non-inoculated plants and inoculated ones could be seen only in pod number on both main stem and lateral shoots. The number of pod on both main stem and lateral shoots were higher in non-inoculated plants than in inoculated ones. Pod number on main stem was not affected by waterlogging treatments, but that on lateral shoot was significantly reduced by waterlogging at the vegetative

Table 3 The effect of Rhizobium inoculation and short-term waterlogging on various characteristics related to seed yield of yard long bean.

Treat-ment[1]	Pod number main stem	Pod number lateral shoots	Seed number per pod	100-seed weight (g)	Pod set rate (%)
N.I.	12.4 A[2]	4.7 A	10.8 A	9.5 A	52.5 A
R.I.	8.7 B	2.5 B	11.9 A	9.1 A	48.9 A
N.W.	9.9 a[3]	5.6 a	10.1 a	9.5 a	49.9 ab
W.V.	10.9 a	0.1 b	12.6 a	9.1 a	42.7 b
W.F.	10.9 a	5.0 a	11.4 a	9.2 a	59.4 a

1) Abbreviations for treatments are the same as those in Fig. 4.
2),3) Different letters indicate significant difference at P=0.05.

stage (Table 3). In seed number per pod and 100-seed weight, no significant effects by waterlogging treatments were observed. Pod set rate was not significantly affected by waterlogging treatments, although it was higher in plants subjected to waterlogging at the flowering stage than in those at the vegetative stage. The significant interaction between the effect of Rhizobium inoculation and waterlogging was not observed in each measured item.

In young pod harvesting, flower number on main stem was not affected by nitrogen application regime (Table 4). It was increased by waterlogging at the vegetative stage, while not affected by the treatment at the flowering stage. In flower number on lateral shoots, the significant interaction between the effect of inoculation and waterlog-

Table 4 The effect of Rhizobium inoculation and short-term waterlogging on flower number in young pod harvesting of yard long bean.

Treatment[1)]	Flower number main stem	Flower number lateral shoots[2)]
N.I.	28.6 A[3)]	55.9 A
R.I.	29.0 A	43.3 A
N.W.	23.6 b[4)]	59.7 a
W.V.	35.1 a	32.4 b
W.F.	27.6 b	56.7 a

1) Abbreviations for treatments are the same as those in Fig. 4.
2) There was a significant interaction between 2 main factors in this item. The value in each treatment plot was as follows (different letters indicate significant difference at P=0.05);
N.I.+ N.W. - 52.0 ab ; R.I. + N.W. - 67.5 a
N.I.+ W.V. - 38.3 bc ; R.I. + W.V. - 26.5 c
N.I.+ W.F. - 77.5 a ; R.I. + W.F. - 36.0 c
3),4) Different letters indicate significant difference at P=0.05.

ging was observed (see footnote 2) in Table 4). There was no significant difference between inorganic nitrogen supplied and inoculated plants except when waterlogged at the flowering stage. When subjected to waterlogging at the flowering stage, flower number on lateral shoots was much more in non-inoculated plants than inoculated ones. There was a tendency that plants waterlogged at the vegetative stage showed fewer flower number than those subjected to the other treatments.

Young pod yield was significantly higher in non-inoculated plants than in inoculated plants (Fig.7). Waterlogging at the vegetative stage had a tendency to reduce young pod yield, but the effect was not statistically significant. The effect of waterlogging at the flowering stage was not found. The interaction between Rhizobium inoculation and waterlogging was not observed.

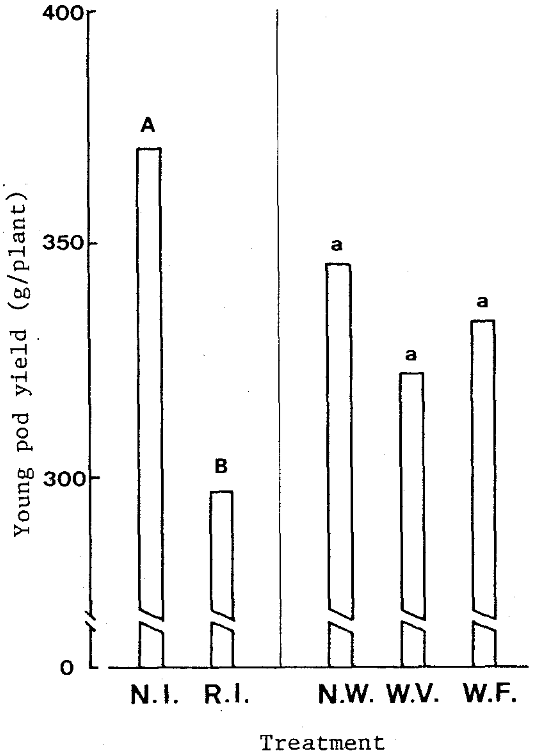

Fig. 7 The effect of Rhizobium inoculation and short-term waterlogging on young pod yield. Abbreviations for treatments are the same as those in the previous figures. Columns covered by the same letter do not differ at the 5 % level.

The effects of Rhizobium inoculation and short-term waterlogging on several traits relating to young pod yield are shown in Table 5. In each parameter, there was no significant difference between inorganic nitrogen supplied and inoculated plants except pod number on lateral shoots. Pod number on lateral shoots in inoculated plants were lower

Table 5 The effect of Rhizobium inoculation and short-term waterlogging on various traits related to young yield of yard long bean.

Treat-ment[1]	Pod number main stem	Pod number lateral shoots[2]	pod weight (g)	Pod set rate (%)
N.I.	19.3 A[3]	31.8 A	7.3 A	62.7 A
R.I.	18.6 A	21.9 B	7.5 A	58.5 A
N.W.	15.5 b[4]	32.1 a	7.3 b	58.0 a
W.V.	22.9 a	17.8 b	8.0 a	58.9 a
W.F.	18.4 b	30.6 a	6.9 b	59.4 a

1) Abbreviations for treatments are the same as those in Fig. 4.
2) There was a significant interaction between 2 main factors in this item. The value in each treatment plot was as follows (different letters indicate significante difference at P=0.05) ;
N.I.+ N.W. - 30.8 ab ; R.I. + N.W. - 33.5 ab
N.I.+ W.V. - 21.8 bc ; R.I. + W.V. - 13.8 c
N.I.+ W.F. - 42.8 a ; R.I. + W.F. - 18.5 bc
3),4) Different letters indicate significant difference at P=0.05.

than that in non-inoculated plants. The effects of short-term waterlogging on pod number on main stem and lateral shoots showed a similar tendency observed in those effects on flower number (Table 5). Pod weight was significantly increased by waterlogging at the vegetative stage, whereas not affected by the treatment at the flowering stage. There were no significant differences among treatments in pod set rate. The significant interaction between Rhizobium inoculation and short-term waterlogging was not observed except pod number on lateral shoots.

Discussion

Both seed and young pod yield in Rhizobium inoculated plants was much lower than that in non-inoculated, inorganic nitrogen supplied ones, resulting from low pod number both on main stem and lateral shoots in seed harvesting and from low pod number on lateral shoots in young pod harvesting. This might be due to low nitrogen supplied by Rhizobium in inoculated plants. Minchin et al. (1978) reported that in cowpea, which belongs to the same species as yard long bean, inoculated plants supplemented with one-tenth amount of inorganic nitrogen as compared to non-inoculated ones could produce a comparable yield to non-inoculated plants supplied with inorganic nitrogen. The reduction of yield in inoculated plants in this experiment might be caused by low application rate of inorganic nitrogen and/or poor affinity of used Rhizobium race for employed cultivar and/or the genotypic differences between cowpea and yard long bean.

The number of days to first flower was not affected by any treatments. The restriction of growth caused by short-term waterlogging may not be sufficient to delay flowering.

The seed yield was significantly reduced by waterlogging at the vegetative stage. Among yield components, only pod number on lateral shoots was reduced by this treatment. This suggests that the yield reduction by waterlogging at the vegetative stage was closely related to the reduction of lateral shoot development, as demonstrated by

Minchin et al. (1978).

The reduction of lateral shoot development might be caused by the competition of assimilates between roots and the other organs and/or imbalance of endogenous plant growth regulators. As shown in Fig.4 and Fig.5, in water-logged plants at vegetative stage, rapid recovery of root growth was observed just after the treatment, while the growth of the above-ground parts was retarded during the same period. This seems to suggest that assimilates were translocated mainly to roots in this period, resulting in the accelerated growth of roots and the restricted growth of the above-ground parts. As the onset of lateral shoot development could be seen in this period in healthy plants, the effect of waterlogging on lateral shoot growth may appear in plants subjected to waterlogging at the vegetative stage. On the other hand, waterlogging treatment is known to reduce biosyntheses of cytokinin (Burrows and Car, 1969) and gibberellin (Reid et al., 1969; Reid and Crozier, 1971) and to increase endogenous level of ethylene (Bradford and Yang, 1981; Kawase, 1972). There is a possibility that these internal regulators are related to poor lateral shoot development in plants subjected to waterlogging to plants at the vegetative stage.

The seed yield was not affected by waterlogging at the flowering stage. Each yield component was not affected either. This is inconsistent to the results obtained in other crops (Cannell et. al., 1979; Krizek, 1981), but consistent to some of other legumes (Minchin et al., 1976;

Sugimoto et al. 1988). The luck of flower abortion and/or the loss of fruit set, which were sometimes observed in other crops damaged by waterlogging at this stage, may explain this inconsistency.

Young pod yield showed a tendency to be lower in plants subjected to waterlogging at the vegetative stage than in those subjected to the other treatments. In young pod yield the effect of short-term waterlogging at this stage appeared to be milder in comparison to that in seed yield. Among various characteristics relating to yield, pod number on main stem and pod weight were increased by this treatment in young pod harvesting, although pod number on lateral shoots were reduced by the same treatment just as the case of seed harvesting. This suggests that the yield reduction caused by poor lateral shoot development was compensated to some extent by the increment of pod number on main stem and weight increase of each pod. Additionally, it is considered that long growing and harvesting period after the treatment allowed plants to recover from the stress induced by waterlogging at the vegetative stage.

Young pod yield was not affected by waterlogging at the flowering stage. Various characteristics relating to the yield were not affected also by this treatment. This might be caused by the same reason as described in seed harvesting.

The responses of yard long bean to short-term waterlogging were different whether plants were waterlogged at the vegetative stage or at the flowering stage. This shows that the sensitivity of plants to waterlogging differs at different growing stages.

In this experiment, the effect of each treatment in young pod yield was very similar to that in seed yield (Fig. 6 and Fig. 7). This suggests that it is possible to predict treatment effect in young pod yield by the result in seed yield. This finding seems to contribute to the actual breeding of yard long bean, because it may enable the screening by seed yield, which is much more efficient than that by young pod yield due to shorter harvesting period and less labor load.

The significant interaction between the effect of short-term waterlogging and *Rhizobium* inoculation was not observed in parameter with some exceptions. As lower nitrogen is supposed to be supplied to *Rhizobium* inoculated plants as compared to non-inoculated, inorganic nitrogen supplied ones, the effect of *Rhizobium* inoculation seems to be confounded with the effect of low nitrogen level in this study. Therefore, it was not clear whether the interaction between the effect of waterlogging and inoculation exists or not. More study is needed on this problem.

Although short-term waterlogging at the vegetative stage reduced the yield, the extent of reduction was much smaller than those in the reported results (Tomooka, 1982; Wien, et al., 1979). This may be related to the sterilization of used soils.

Section 2. Effects of waterlogging duration

Introduction

Duration of soil submersion is one of the major factors which influence the plant response to waterlogging. It is generally considered that the longer the waterlogging period, the more adversely plants are affected. Large decreases in yield after only one day of waterlogging have been reported in many legumes including pea (Cannell et al., 1979; Jackson M.B., 1979), cowpea (Minchin et. al., 1978), bean (Forsythe et al., 1979) and soybean (Wien et al., 1979). On the other hand, some of upland crops can survive under waterlogging conditions for a long term (Ruegg, 1981).

In order to clarify the effect of the length of the waterlogging period, the following experiment was carried out. As in the experiment in the former section, soils were sterilized by fumigation before use to minimize the interaction between plant roots and soil microorganisms. Additionally, nutrient solution was used for submerging water so that deficiency of nutrient was not the limiting factor for the growth.

Materials and Methods

Yard long bean plants, cv. 'TKC-83' were sown in vermiculite on May 2nd in 1988. Ten days later they were transplanted in 24 cm diameter clay pots filled with a mixture of sand and organic manure at the ratio of 2:1. Plants were grown in a plastic greenhouse without control of the temperature. Proper amount of nutrient solution (half strength of 'Enshi' solution, Yamazaki, 1982) was supplied as needed. Changes in air temperature during this experiment are shown in Fig.8.

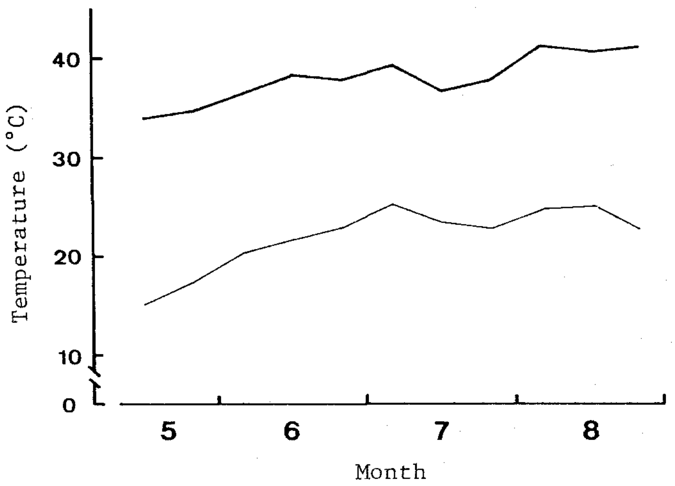

Fig. 8. The fluctuation of ambient maximum (—) and minimum (–) temperature during the experiment.

Waterlogging treatment was applied by placing the pots into plastic buckets and filling the buckets with the nutrient solution. The water surface was kept at 2 cm above the soil level by adding proper amount of nutrient solution. The surface of each pot was covered with black cheese cloth in order to prevent algae proliferation. Appropriate number of plants were subjected to waterlogging for 4 days (short-term treatment), 16 days (long-term treatment) and all the remaining period until harvest (continuous treatment). The treatment began at 28 days after sowing when 2-3 trifoliate leaves were expanded.

Experiment for yield determination was conducted as a randomized block design with 10 replications. Plants were spaced 30 cm in-row and 70 cm between-row. In this experiment, only the seed yield was measured, because it is much easier to measure this parameter than the pod yield and it was found that there was a high correlation between the effect of waterlogging on seed yield and that on pod yield (see the previous section). Several characteristics relating to yield were also determined.

Five plants in each treatment plot, separately grown at the same plant spacing in the same plastic greenhouse, were sampled at 0, 4, 16 and 28 days after the beginning of the treatment. Root system of each plant was carefully observed with naked-eyes. All sampled plants were then separated into their various components and fresh and dry weights were measured.

Results

In plants subjected to waterlogging, hypertrophy of stem base was observed around water surface. In such a swollen stem, the epidermis and the cortex were destroyed and white and spongy tissue was established. Even in the short-term treatment plot, such tissue was developed and after the withdrawal of the treatment, the surface of this tissue was dried. In plants subjected to long-term and continuous waterlogging, this tissue was further developed. Primary root system in the soil decayed gradually and finally rotted at the time of the last sampling. On the other hand, adventitious roots were initiated on the white and spongy tissue on 5 to 6 days after the beginning of the treatment and developed abundantly and vigorously afterward. Most of those adventitious roots are located just near the water surface. Upon the removal of the stress they are exposed directly to air and dried rapidly in the long-term treatment plot. The development of another adventitious roots was observed thereafter in this plot. In plants subjected to continuous waterlogging, vigorous adventitious root development continued up to the final sampling date.

Fig. 9 and Fig. 10 show the effects of waterlogging with various duration on the growth of the above-ground parts and roots, respectively. Short-term waterlogging retarded the growth of both the above-ground parts and roots during the treatment, but rapid recovery, especially in roots, was observed soon after the termination of the treatment. The long-term treatment restricted the growth of the above-ground parts until the 16th day after the beginning of the treatment (the day when the treatment was completed). Roots recovered from the damage which was observed after the first 4 days of the treatment. Post-stress growth of plants subjected to long-term waterlogging was restricted in both the above-ground parts and roots. On the other hand, both the above-ground parts and roots showed further recovery in the continuous treatment plot.

Seed yield was slightly reduced by short-term and continuous waterlogging without statistical significance (Fig. 11). The yield was reduced to about 75 % of the

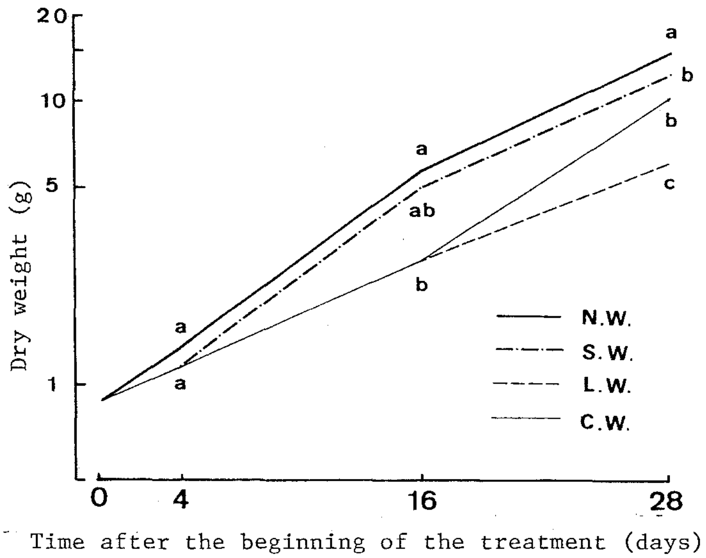

Fig. 9. The effect of waterlogging duration on the changes in dry weight of above-ground parts. N.W.; non-waterlogged, S.W.; subjected to short-term waterlogging, L.W.; subjected to long-term waterlogging, C.W.; subjected to continuous waterlogging. Different letters at each measurement time indicate significant difference at p=0.05.

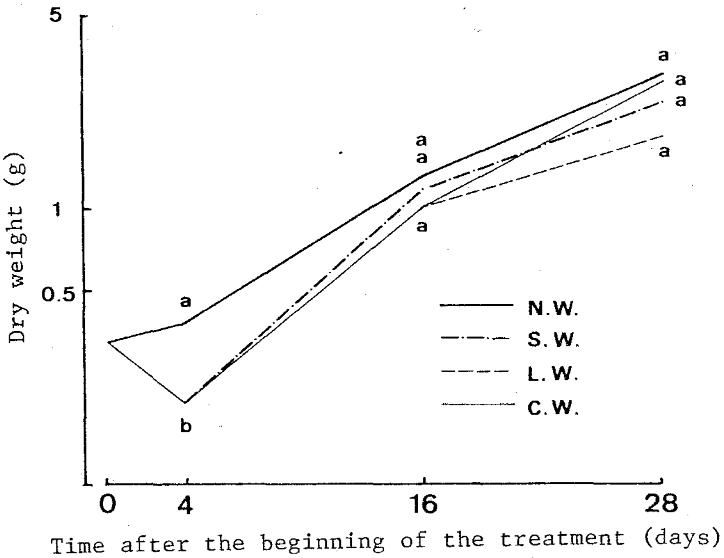

Fig. 10. The effect of waterlogging duration on the changes in dry weight of roots. Abbreviations for treatments are the same as those in the previous figures. Different letters at each measurement time indicate significant difference at p=0.05.

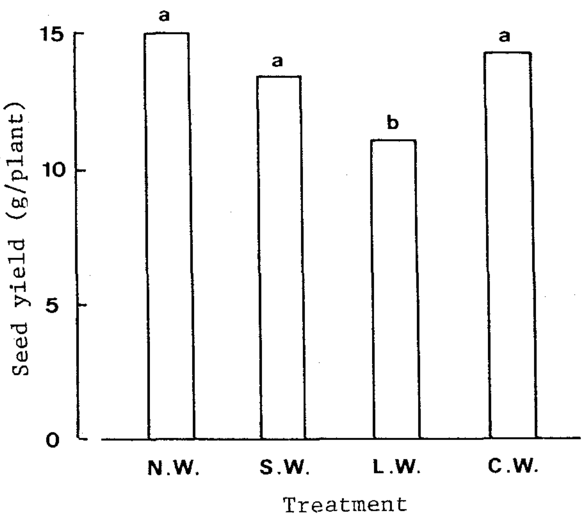

Fig. 11. Effect of waterlogging duration on the seed yield. Different letters indicate significant difference at p=0.05. Abbreviations for treatments are the same as those in Fig. 9. Columns covered by the same letter do not differ at the 5 % level.

control by the long-term treatment.

The number of days to first flower was not affected by short-term waterlogging (Table 6). Flowering was delayed significantly by the long-term treatment and slightly by the continuous treatment. The number of flowers on main stem was not affected significantly by waterlogging treatments, although it had a tendency to be reduced by the long-term and the continuous treatment. The number of flowers on lateral shoots was reduced significantly by long-term waterlogging.

Table 7 shows the effects of waterlogging on various characteristics relating to yield. The number of pod on main stem was not affected significantly by waterlogging treatments, but that on lateral shoot had a tendency to

Table 6 The effect of waterlogging duration on the number of days to first flower in yard long bean.

Treatment	Number of days to first flower (days)
Control	56.8 b*
Short-term	57.0 b
Long-term	64.0 a
Continuous	59.6 b

* Different letters indicate significant difference at P=0.05.

Table 7. The effect of waterlogging duration on various characteristics relating to seed yield.

Treatment	Number of pods Main stem	Number of pods Lateral shoot	Number of seeds per pod	100-seed weight (g)	Pod set rate (%)
Control	9.1 a*	4.4 ab	10.0 ab	10.8 a	46.8 b
Short-term	9.6 a	3.9 ab	9.9 b	10.0 b	50.2 ab
Long-term	8.3 a	1.2 b	11.1 ab	9.9 b	54.4 ab
Continuous	9.0 a	4.8 a	11.6 a	8.4 c	58.5 a

* Different letters indicate significant difference at p=0.05.

be lowered by long-term waterlogging, although significant difference was only found between the long-term treatment plot and the continuous treatment plot. The number of seed per pod was the greatest in the continuous treatment plot and significantly larger in this plot than in the short-term treatment plot. One hundred seed weight was reduced by waterlogging treatments and was the smallest in plants subjected to continuous waterlogging. Pod set rate had a tendency to increase by waterlogging and was significantly larger in the continuous treatment plot than in control plot. The number of productive lateral shoots was slightly reduced by the long-term and continuous treatments without statistical significance.

Discussion

The results obtained here indicate several interesting aspects. Firstly, yard long bean cv. 'TKC-83' was not only able to survive under the continuous waterlogging conditions but also the yield was comparable to that of the non-stressed control, although the retardation of growth for some period and slight delay of flowering were observed. This fact reveals that if influences by soil microorganisms are minimized and proper nutrients are supplied, yard long bean plants can adapt themselves to waterlogging conditions and leads to the necessity to reconsider the role of soil microorganisms in waterlogging damage. Although further studies should be carried out, the incorporation of the activities of soil microorganisms into plant response to waterlogging should not be overlooked.

The survival and vigorous regrowth observed in plants subjected to continuous waterlogging may be presumably related to the development of the white and spongy tissue and adventitious roots. The white and spongy tissue, which was formed at stem base around water surface in plants subjected to waterlogging, looked similar to the tissue observed in a waterlogged wild soybean (Arikado, 1975).

Arikado (1975) made a careful anatomical analysis and concluded that this tissue played a role of secondary aerenchyma. Further morphological studies on this tissue were conducted in the next chapter.

There is controversy as to whether adventitious root development is a symptom of flooding damage or a beneficial adaptation to waterlogging (Krizek,1981; Kawase, 1981). The result obtained here clearly suggested that this characteristics was a useful trait for plants to be adapted to waterlogging conditions, because the recovery of the growth was observed after the formation of adventitious roots. At harvesting time, plants subjected to continuous waterlogging were completely adapted to the environment, and growing like plants in hydroponics. Perhaps, careful management of the nutritional state of submerging water will increase the yield of continuously submerged plants.

Secondly, the long-term treatment in this experiment restricted the growth and yield in comparison to the other 2 treatments. It was shown that once yard long bean plants became adapted to the waterlogging conditions, the discontinuation of the treatment resulted in adverse effects on the plants. Because most of the active adventitious roots are located near the water surface, they are directly exposed to air upon the removal of the stress and become useless for water and nutrient uptake. Thus if prolonged waterlogging is discontinued, plants are subjected to dual stress, that is the first stress caused by soil anaerobiosis and the second caused by the removal of the stress after the adaptation to the anaerobic conditions. Even if the waterlogging period is short, plants are supposed to be subjected to this dual stress, but usually the adaptation of plants is not complete in this case and the damage caused by the second stress is not very severe.

The yield reduction in plants subjected to long-term waterlogging seemed to be related to the reduction of pod number on lateral shoot. Though 100-seed weight was also decreased by the long-term treatment, this was compensated by the increase of the number of seed per pod. In plants subjected to continuous waterlogging, the similar phenomenon was observed. Seed size was reduced, but it was compensated by the increase of the number of seeds per pod.

Short-term waterlogging reduced seed yield slightly without statistical significance. This was not in agreement with the result in the previous section. The growth of lateral shoot development was not retarded in this experiment. In the former section, the difference of the growth pattern between the above-ground parts and roots was considered to be the major cause of the restriction of lateral shoot development. As the growth pattern of the above-ground parts and roots during and after the treatment was quite similar to that observed in the former experiment, subtle changes of ambient conditions may have affected the timing of the onset of lateral shoot development and the effects of short-term waterlogging on this character seem to have disappeared. The period, when yard long bean plants are sensitive to growth retardation caused by waterlogging, may not be long.

Section 3. Effects of soil sterilization on the responses of yard long bean to waterlogging

Introduction

In the previous sections, the possibility was suggested that indirect effects of soil waterlogging, especially the activities of soil microorganisms, play a large role in the response of yard long bean to waterlogging. In this section, the experiment was designed to evaluate the indirect effects of waterlogging by comparing the response of yard long bean plants to continuous waterlogging, grown in non-sterilized and sterilized soils.

Materials and methods

Yard long bean plants, cv. 'TKC-83' were sown in vermiculite on June 13th in 1989. Ten days later they were transplanted in 24 cm diameter clay pots filled with orchard soil, rich in organic matter. Fluctuation of ambient temperature during this experiment is shown in Fig. 12. Proper amount of nutrient solution (half strength of 'Enshi' solution, Yamazaki, 1982) was supplied as needed.

Half of the soil was sterilized by fumigation before use. The remaining half was not applied to any treatments. At the time of transplanting, whole plants were divided into two groups. Plants in one group were transplanted in pots containing sterilized soils and plants in the other group were transplanted in pots in non-sterilized soils.

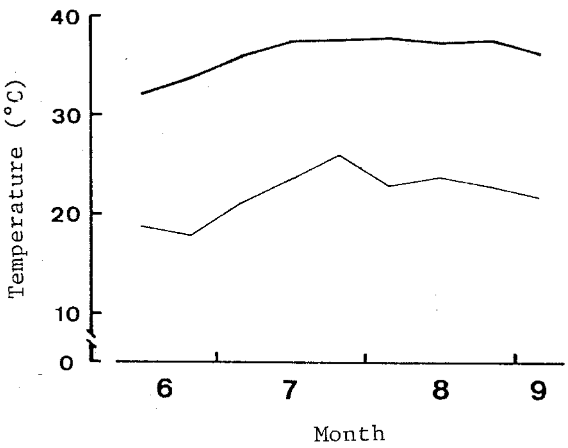

Fig. 12. The fluctuation of ambient maximum (–) and minimum (-) temperature during the experiment.

Waterlogging treatment was applied in the same manner as those described in the former section. Appropriate number of plants were subjected to waterlogging for the remaining period until harvest. The treatment began at 28 days after sowing when 2-3 trifoliate leaves were expanded.

Experiment was conducted as a randomized block design with 12 replications. Plants were spaced 30 cm in-row and 70 cm between-row. On 0, 4, 6, 8, 10, 12, 14, 16, 20, 24 and 28 days after the beginning of the treatment, the number of leaves, length of 3 to 6 developing leaves (length between the end of the petiole and the top of the apical leaflet) and chlorophyll contents of matured leaves were measured. Leaf chlorophyll contents were measured by a Green meter (Fuji Film Co. Ltd.). Relative values measured by this machine were converted to chlorophyll contents by a standard curve calculated by the regression of measured values to actual chlorophyll contents. After harvesting pods, seed yield and several characteristics relating to yield were determined.

Results

As the significant interaction between 2 main factors was observed in many of the measured items, data in each experimental plot were shown in the figures and tables here.

In both non-sterilized and sterilized soils, non-waterlogged plants could grow well. Some of waterlogged plants grown in non-sterilized soils could not survive until the termination of the experiment, while all waterlogged plants grown in sterilized soils could survive. As indicated in the previous section, waterlogged plants formed white and spongy tissue and developed adventitious roots, although such tissue and adventitious roots formation were retarded to some extent in several plants grown in non-sterilized soils. In plants, which could not survive, the stem showed severe necrosis. Among plants which dared to survive, some showed chlorosis in upper leaves and browning of adventitious roots. Several others showed only browning of adventitious roots.

Fig. 13 shows the effect of continuous waterlogging and soil sterilization on the changes in leaf number. Non-waterlogged plants, irrespective of soil sterilization, showed a steady increase of leaves and reached the maximum leaf number on 20 to 24 days after the beginning of the treatment. At this time, most of non-waterlogged plants

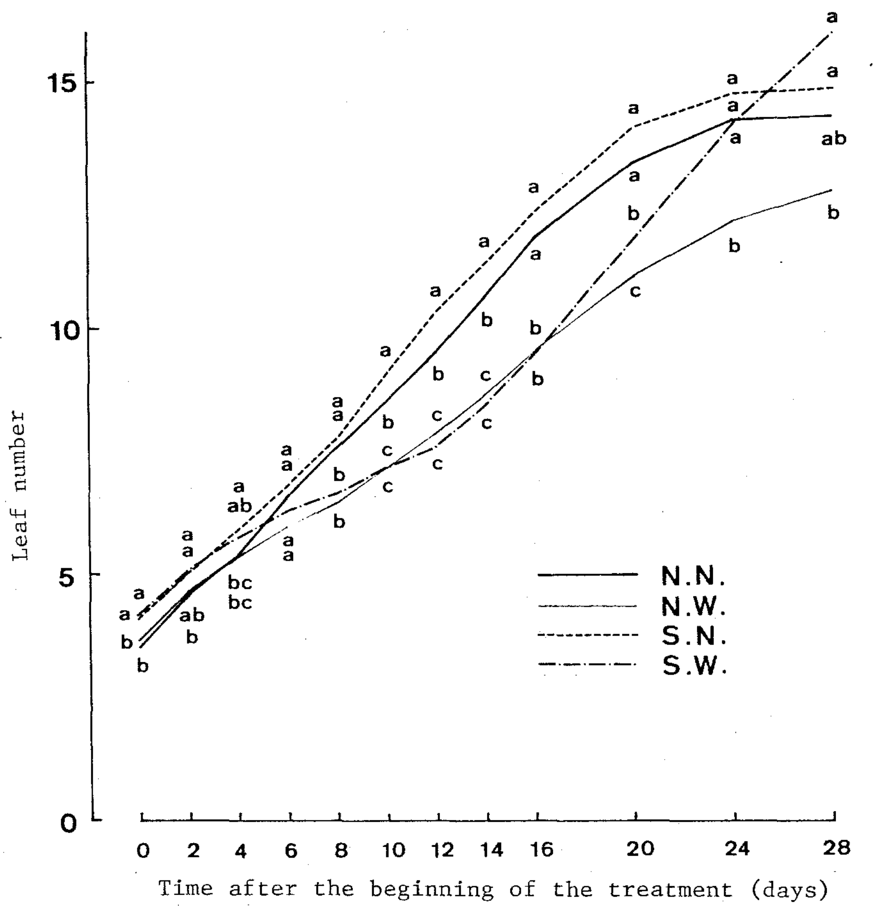

Fig. 13. The effect of continuous waterlogging and soil sterilization on the changes in leaf number. N.N.; non-sterilized and non-waterlogged, N.W.; non-sterilized and waterlogged, S.N.; sterilized and non-waterlogged, S.W.; sterilized and waterlogged. Different letters at each measurement time indicate significant difference at p=0.05.

began to flower and stopped the expansion of new leaves. On the other hand, in plants subjected to continuous waterlogging, reduction of leaf number was observed on 6 days after the beginning of the treatment, in both non-sterilized and sterilized soils. This tendency continued until 16 days after the onset of waterlogging. Thereafter plants grown in sterilized soils increased leaf number rapidly and were still continuing new leaf expansion after non-waterlogged control plants stopped leaf growth. On the contrary, most of waterlogged plants grown in non-sterilized soils showed relatively slow increase of leaf number until the final measurement date.

Fig. 14 shows the influences of waterlogging in different soil conditions on leaf length. In plants grown in non-sterilized soils, the decrease of length caused by waterlogging was observed in the leaves on upper than the 4th node. The extent of reduction was most in the 5th to 7th leaves, growing period of which was synchronized with 6 to 16 days after the onset of waterlogging. In plants grown in sterilized soils, leaf length in 4th to 8th leaves was reduced by waterlogging. The extent of reduction was most in the 6th and 7th leaves. This was also synchronized with the growing period of 6 or 8 to 16 days after the beginning of the treatment. Length of leaves on upper than 9th node became comparable to or even longer than control plants.

The chlorophyll contents of newly matured leaves were reduced by waterlogging treatment in both non-sterilized and sterilized soils, but the degree of reduction was more severe in plants grown in non-sterilized soils (Fig. 15). The difference of the degree of reduction became remarkable after 6th day. Plants subjected to waterlogging in sterilized soils showed rapid recovery in chlorophyll contents after 16th day, while most of those in non-sterilized soils maintained low level of leaf chlorophyll contents.

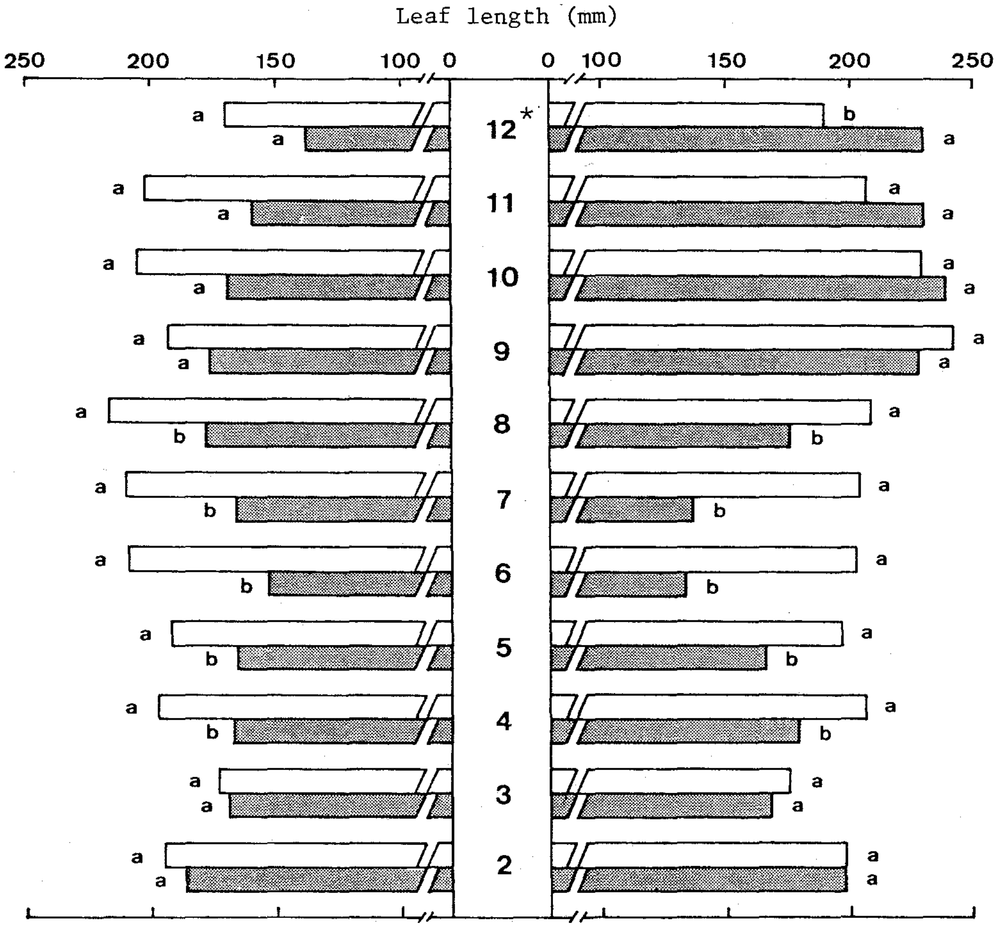

Fig. 14. The effect of continuous waterlogging and soil sterilization on leaf length. Left side; non-sterilized, right side; sterilized, *; node number. Open bar indicates non-waterlogged plants and dotted bar waterlogged ones. Bars with the same letter do not differ at the 5 % level.

Table 8 shows the effect of waterlogging and soil sterilization on the number of days to first flower. As stated before, some of waterlogged plants grown in non-sterilized soils could not survive and died before flowering. Thus data on such plants were excluded in the result shown in Table 8. In this parameter, the significant inter-

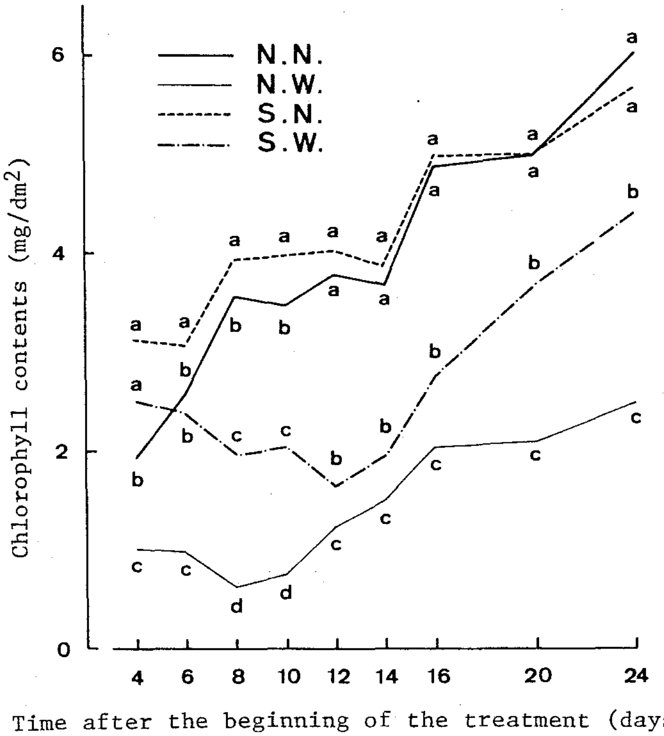

Fig. 15. The effect of continuous waterlogging and soil sterilization on the changes in leaf chlorophyll contents. Abbreviations for treatments are the same as those in the previous figures. Different letters at each measurement time indicate significant difference at p=0.05.

Table 8. The effects of waterlogging and soil sterilization on the nuber of days to first flower

Treatment	Number of days to first flower (days)
N.N.[1)]	45.4 b[2)]
N.W.	49.6 a
S.N.	42.8 c
S.W.	47.2 b

1) Abbreviations for treatments are the same as those in Fig.13.
2) Different letters indicate significant difference at P=0.05.

action between main factors was not found. Soil sterilization shortened the number of days to first flowers and waterlogging delayed flowering.

The seed yield was reduced by continuous waterlogging in plants grown in non-sterilized soils, while not reduced significantly in those grown in sterilized soils (Fig. 16).

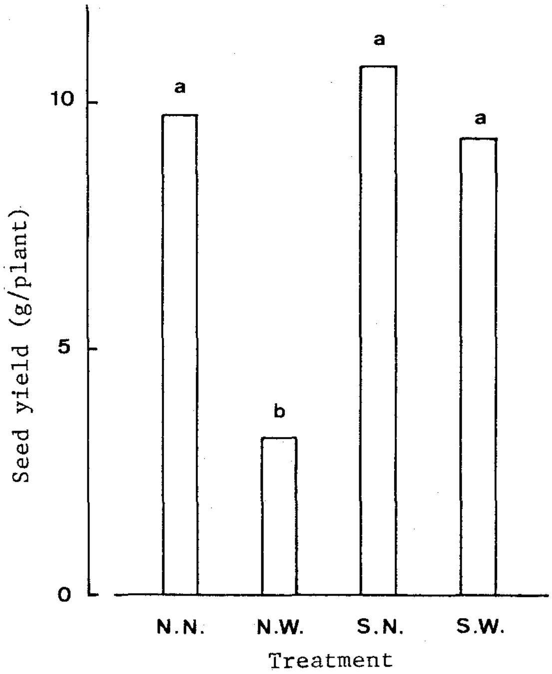

Fig. 16. The effect of continuous waterlogging and soil sterilization on the seed yield. Abbreviations for treatments are the same as those in Fig. 13. Columns covered by the same letter do not differ at the 5 % level.

Table 9 shows the effects of waterlogging and soil sterilization on various characteristics relating to yield. The number of pod in main stem showed a similar tendency to the yield. The number of pod in lateral shoots was nearly zero in all experimental plots. In this experiment, the development of lateral shoots was restricted even in control. Almost no productive lateral shoots were found in all plots either. Seed number per pod was reduced by waterlogging in non-sterilized soils, whereas increased slightly in sterilized soils. One hundred seed weight showed a similar tendency to the yield.

Table 9. The effect of soil sterilization and waterlogging on various traits relating to yield.

Treatment[1)]	Pod number main stem	Pod number lateral shoots	Number of seeds per pod	100-seed weight (g)
N.N.	8.3 a	0 a	10.0 b	11.9 a
N.W.	3.1 b	0.3 a	6.8 c	6.0 b
S.N.	8.1 a	0.3 a	11.5 ab	11.6 a
S.W.	7.2 a	0.2 a	13.9 a	10.0 a

1) Abbreviations for treatments are the same as those in Fig. 9.
2) Different letters indicate significant difference at P=0.05.

In most of waterlogged plants grown in non-sterilized soils showed poor growth and yield, but two of 12 replications could recover vigorously just like waterlogged plants grown in sterilized soils. The yield in those plants was comparable to that in control plants (14.59 and 7.47 g/plant respectively).

Discussion

Although primary cause of waterlogging damage is considered to be root oxygen deficiency (Jackson, 1979; Krizek, 1981), the result of this experiment showed that indirect effects of soil waterlogging other than root oxygen deficiency was also very large in the response of yard long bean to the stress.

In both non-sterilized and sterilized soils, retardation of the growth of the above-ground portions began to appear on 4 to 6 days after the beginning of the treatment and continued up to about 16th day, as indicated by the results on changes of leaf number, leaf length and changes of leaf chlorophyll contents. Waterlogged plants grown in sterilized soils showed rapid recovery afterwards. This is in agreement with the results obtained in the previous experiment. During the period of growth limitation of above-ground parts the development of adventitious roots was observed. On the other hand, although the development of adventitious roots was found to some extent, waterlogged plants grown in non-sterilized soils did not show further recovery with some exceptions. Such plants, including those which died before flowering and survived up to harvest showed various symptoms described in the results. These symptoms seemed to be caused by pathogenic infection of both fungi and bacteria and/or the accumulation of toxic materials (Fitter and Hey, 1981). All of them appear to be resulted from indirect effects of waterlogging, which are considered to be mediated by the activities of soil microorganisms (Fitter and Hey, 1981; Skinner, 1975).

It is generally accepted that the indirect effects of waterlogging appear after rather long period of soil submersion (Jackson, 1985). But the result on the changes of leaf chlorophyll contents showed that plants waterlogged in non-sterilized soils began to be affected by the treatment more adversely than those in sterilized soils even shortly after the onset of waterlogging. High temperature during the treatment period may activate soil anaerobic microorganisms faster, resulting in the early appearance of the indirect effects.

Seed yield was severely reduced by continuous waterlogging in plants grown in non-sterilized soils. Most of plants which dared to survive bare very few pods, resulting in poor yield. This may be due to poor growth caused by the above- mentioned factors. On the contrary, the yield of waterlogged plants grown in sterilized soils was comparable to that of non-waterlogged control plants, consistent to the result in the former section.

The present results clearly indicated that yard long bean, 'TKC-83', had an adaptability to root oxygen deficiency, but that it was very susceptible to some of indirect effects of waterlogging. It was also proved how large the role of indirect effects of waterlogging was in plant responses to the stress.

Seed yield in this experiment was generally low in comparison to the previous experiments. Lateral shoot development was retarded even in non-waterlogged control. This may be caused by the high temperature during the experiment. High temperature especially during the vegetative stage accelerates reproductive development of lateral buds and inhibits lateral shoot formation (Hadley et al., 1983; Summerfield and Roberts, 1983).

Several plants grown in non-sterilized soils could survive and resume vigorous growth. The same phenomenon was observed in winged bean (Ruegg, 1981). Waterlogging induces the activation of soil anaerobic microorganisms which cause various chemical reactions, resulting in the accumulation of toxic substances. It also induces the proliferation of pathogenic fungi and bacteria. On the other hand, some of useful anaerobic bacteria for plant growth such as Clostridium are also activated by waterlogging. Among those microorganisms, some may become dominant and affect plant growth variously. Which bacteria or fungi dominate or which reactions mainly occur are dependent on the complicated interaction between the activities of each microorganisms and soil environment. Even if soils from the same source are used, dominant microorganisms or dominant reactions which occur in the soils are not always the same because of the difference of micro-environment. Thus some of waterlogged plants grown in non-sterilized soils could be adapted to the environment and grow well. If this phenomenon occurred as the result of dominance of useful microorganisms, it might exert the possibility of biological control of the stress. Further study is necessary on this matter.

Section 4. Conclusion

The experiments conducted in this section reveal the several aspects concerning the responses of yard long bean to waterlogging.

Firstly, because the experiments were conducted under the conditions in which the influences of microorganisms are minimized except the experiment on the effect of soil sterilization, direct effects of root oxygen deficiency on plants could be evaluated. As the results, various factors are proved to affect the responses of yard long bean to root oxygen deficiency. Those factors include the stage of growth, Rhizobium inoculation and duration of the stress.

Waterlogging for short period at the vegetative stage reduced the growth and yield of yard long bean in the experiment in the section 1, presumably due to the restriction of lateral shoot development, while that at the flowering stage gave a limited influence to the growth and yield. These facts may indicate that there is a period when the plants are susceptible to the stress. The same treatment at the vegetative stage, however, affect the growth and yield to much less degree in the experiment in the section 2. It is considered that subtle changes in environmental conditions interact with the susceptibility of the plants.

Rhizobium inoculation did not affect the plant response to short-term waterlogging. It is difficult to conclude that there is no interaction between the response of yard long bean to short-term waterlogging and Rhizobium inoculation from this result, because the effect of Rhizobium inoculation appears to be confounded with the effect of low nitrogen level. Root nodulation is known to be inhibited by root anaerobiosis (Minchin and Pate, 1975; Krizek, 1981). More study is needed on this problem.

Waterlogging duration affected the growth and yield of yard long bean differently. When short-term waterlogging was removed, the plants showed rapid recovery especially in roots. If waterlogging prolonged, yard long bean plants could adapt themselves and resume growth after short-period of growth retardation. When waterlogging was terminated after the completion of the adaptation, post-stress growth was retarded and the yield was reduced. Thus waterlogged plants are subjected to dual stress, i.e. the first stress associated with soil anaerobiosis and the second associated with the removal of the stress. Even if the waterlogging duration is short, plants are subjected to the dual stress. In this case, however, usually the adaptation of the plants is not sufficient and the plants are less affected by the second stress.

Secondly, damages caused by root oxygen deficiency were much smaller than those expected from the observations in actual field conditions and reported results (Tomooka 1982; Wien et al., 1979). Although several waterlogging treatments reduced the growth and yield of plants grown in sterilized soils, the degree of the suppression was not large. Even if plants were affected adversely by waterlogging, they usually showed rapid recovery afterward. These results seem to indicate that yard long bean is tolerant to root oxygen deficiency to some extent, in spite of the established fact that this species is susceptible to waterlogging. As stated previously, waterlogged plants received 2 different effects, i.e. direct effects of root oxygen deficiency and indirect effects other than root oxygen deficiency, which are mainly mediated by the soil microorganisms (Fitter and Hey, 1981). The results of the experiment in the last section clearly showed that yard long bean is susceptible to some of indirect effects of waterlogging caused by other factors than root oxygen deficiency.

Chapter 3. Morphological and physiological changes caused by soil waterlogging

Section 1. Observation of hypertrophied stem tissue

Introduction

Soil inundation induces several morphological changes of plants, including reduced stem elongation, senescence, abscission of the lower leaves, wilting, stem hypertrophy (swelling), leaf epinasty, leaf chlorosis and necrosis, aerenchyma and lenticel formation and adventitious root development (Kawase, 1981). The degree of morphological changes seems to depend on the severity of stress itself and plant genotype. Some of those morphological changes may simply show the damages caused by waterlogging, and some of them may have adaptive significance to the environment.

Among those morphological changes, aerenchyma (air space) formation in the cortex of roots and stem are considered to be closely related to the ability of plants to survive under waterlogging conditions (Arikado, 1953, 1954, 1955; DeWit, 1978; Kawase, 1979, 1980, 1981; Yamasaki, 1955). Rice, which can grow well under flooding conditions, has developed aerenchyma in stem and roots (Arikado, 1953; Yamasaki, 1955). Various hydrophytic plants grown in wet areas are also known to develop ventilating system in their stem and roots (Arikado, 1959; Teal and Kanwisher, 1966; Yamasaki, 1955). Aerenchyma formation under waterlogging conditions was reported to occurs in mesophytic Gramineae, such as wheat (Dunn, 1921), barley (Bryant, 1934) and corn (Drew et al., 1979; Dunn, 1921; McPherson, 1939), and was also observed in other herbaceous mesophytes, including sunflower, tomato, Brussels sprout, cabbage and mustard (Kawase, 1980, 1981), and eggplants and cucumbers (Inden, 1956). Development of ventilating systems in marsh plants and aerenchyma formation in upland plants when subjected to waterlogging appear that the existence of internal air spaces plays an important role for plants to be adapted to waterlogging conditions. These systems may help oxygen transport from aerial parts to roots for the improvement of root oxygen deficiency.

In the experiments described in the previous chapter, hypertrophy of stem base and the formation of white, spongy tissue were observed in waterlogged plants irrespective of the waterlogging duration. Arikado (1975) suggested that similar tissue found in a wild soybean grown under waterlogging conditions included well developed aerenchyma and was contributed to its ability to survive under such conditions. In the following experiment, anatomical observation of hypertrophied stem tissue of yard long bean plants subjected to waterlogging was carried out and its function was considered. As in the experiments in the former chapter, soils were sterilized by fumigation before use to minimize the interaction between plant roots and soil microorganisms.

Materials and methods

Yard long bean plants, cv. 'TKC-83', were sown on June 6th in 1989 and transplanted 13 days later. Cultural practices and the treatments were the same as those described in the previous chapter. Fluctuation of ambient temperature during this experiment and that in the next section is shown in Fig. 17. Appropriate number of plants were subjected to waterlogging for 4 days (short-term treatment) and all the remaining period until the termination of the experiment (continuous treatment). The treatment began at 29 days after sowing when 2-3 trifoliate leaves were expanded.

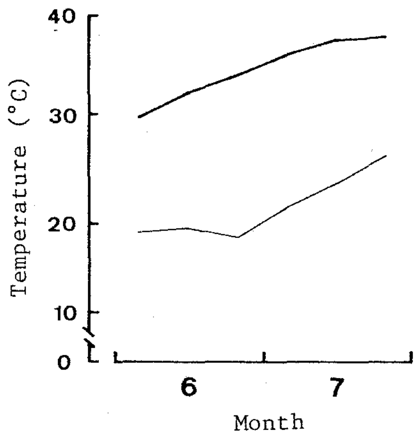

Fig. 17. The fluctuation of ambient maximum (–) and minimum (-) temperature during the experiment.

Several sections, approximately 3 mm thick, sliced at the basal parts of stem were sampled, on 0 (the day when the treatment was started), 2, 4 (the day when the short-term treatment was ended), 8 and 12 days (the day when the experiment was terminated) after the onset of the treatment. The samples were prefixed with FAA (a mixture of formaline, acetic acid and 50 % ethyl alcohol at the ratio of 1:1:18) and stored at 4°C until all the samples were collected. The samples were then refixed firstly by 2 % gultal aldehyde and secondly by 1 % osmium tetraoxiside. Fixed samples were dehydrated by various concentration of ethyl alcohol. The samples were then dried by a critical point dryer ('HCP-2', Hitachi Co. Ltd.). Dried samples were fastened to aluminum stubs using graphite paste. The samples and stubs were gold-coated on an sputter coater ('EIKO IB-3', Akashi Co. Ltd.) and examined on an scanning electron microscope ('Akashi Alpha-10', Akashi Co. Ltd.).

Results

Fig. 18 and Fig. 19 shows transverse sections of the basal part of a stem in control plants on the day of the beginning of the treatment. Well developed central cavity, xylem, phloem, cambium and cortex were observed. In cortex, aerenchyma formation was not found.

Two days of waterlogging induced formation of small internal air spaces in cortex (Fig. 20 and Fig. 21). Rudimental cell walls were observed in those air spaces.

On 4 days after the beginning of the treatment, some part of cortex began to be destroyed, and bigger aerenchyma was developed (Fig. 22 and Fig. 23).

On 8 days after the onset of waterlogging, additionally to the destruction of cortex, breakdown of epidermis was also observed and bare cell walls were exposed directly to the air spaces in plants subjected to the con-

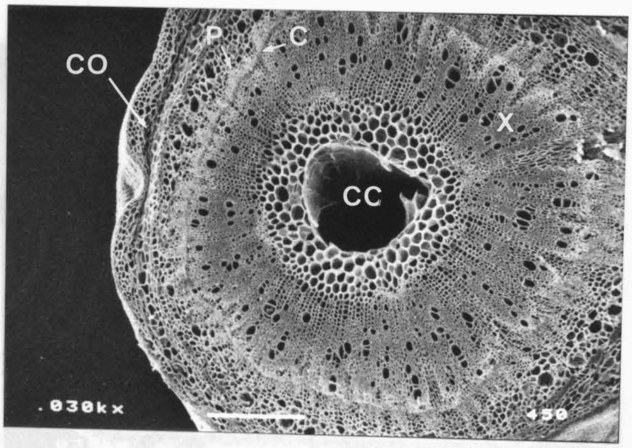

Fig. 18. Transverse section of the basal part of a stem in non-waterlogged plants on the day of the onset of waterlogging (x30). CC; central cavity, X; xylem, P; phloem, C; cambium, CO; cortex.

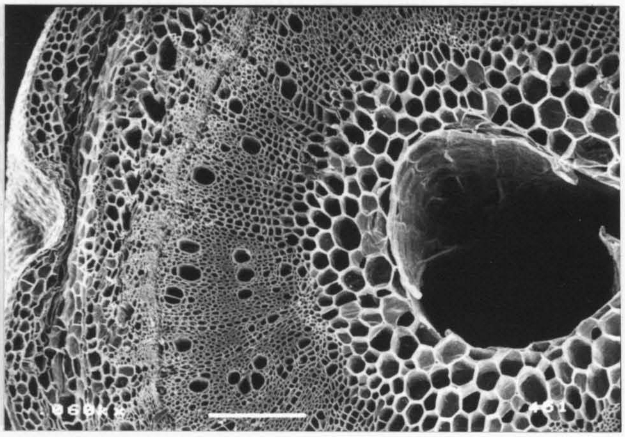

Fig. 19. Transverse section of the basal part of a stem in non-waterlogged plants on the day of the onset of waterlogging (x60).

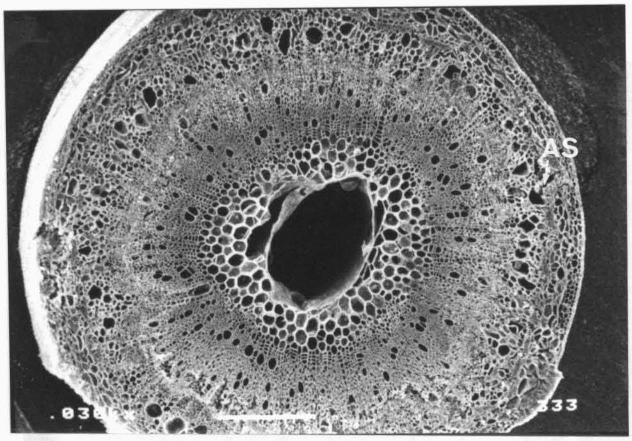

Fig. 20. Transverse section of the basal part of a stem in waterlogged plants on 2 days after the onset of waterlogging (x30). AS; air space.

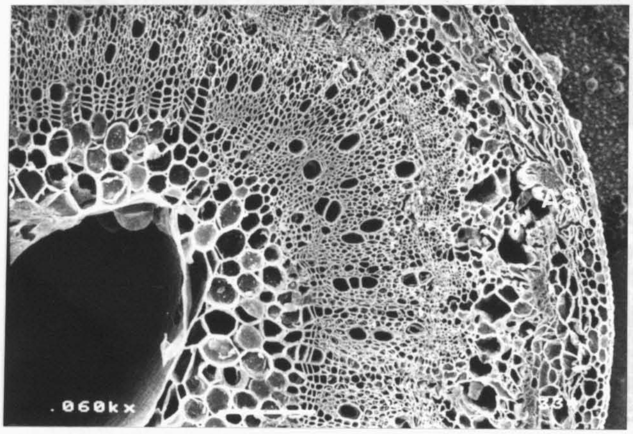

Fig. 21. Transverse section of the basal part of a stem in waterlogged plants on 2 days after the onset of waterlogging (x60). AS; air space.

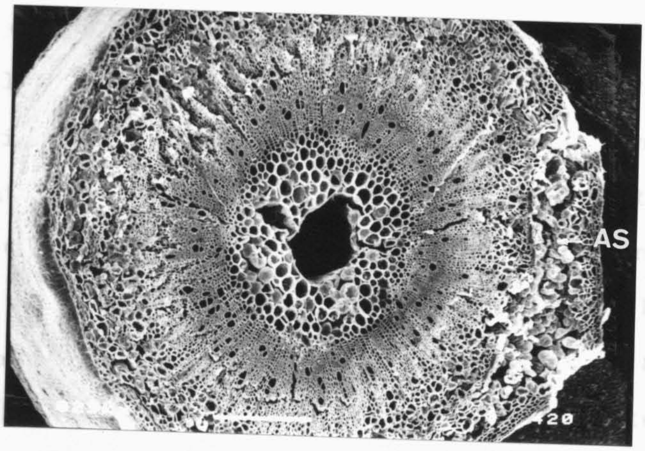

Fig. 22. Transverse section of the basal part of a stem in waterlogged plants on 4 days after the onset of waterlogging (x30). AS; air space.

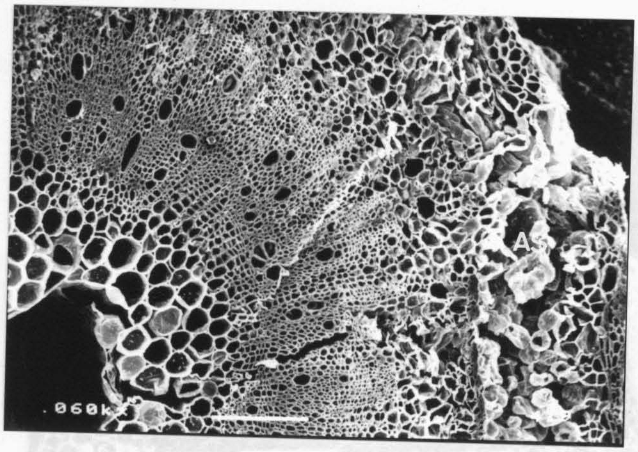

Fig. 23. Transverse section of the basal part of a stem in waterlogged plants on 2 days after the onset of waterlogging (x60). AS; air space.

tinuous treatment (Fig. 24 and Fig. 25). Even if waterlogging was withdrawn on 4 days after the beginning of the treatment, the development of aerenchyma and destruction of cortex and epidermis were going on (Fig. 26 and Fig. 27). Exposed cell walls were also observed (Fig. 27).

Fig. 28 and Fig. 29 shows cross sections of basal stem of control plants on 13 days after the onset of waterlogging. Constitutional factors of a section were not changed in comparison to those observed just before the beginning of the waterlogging treatment (Fig. 18 and Fig. 19). In plants continuously subjected to waterlogging, large air spaces were formed in cortex, and some part of epidermis was completely destroyed (Fig. 30 and Fig. 31). Compared to control plants, central parts were not changed

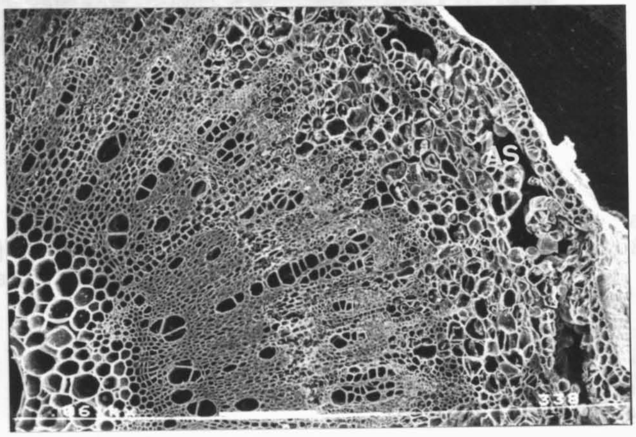

Fig. 24. Transverse section of the basal part of a stem in waterlogged plants on 8 days after the onset of waterlogging (x60). AS; air space.

and only cortical portions were destroyed and swollen. In control plants, a few layers of cell were found in cortex, while in waterlogged plants more than 10 layers of cell were observed in swollen cortex. In plants subjected to the short-term treatment, cortical parts were contracted and the destroyed epidermis was dried (Fig. 32 and Fig. 33). Internal air spaces became smaller.

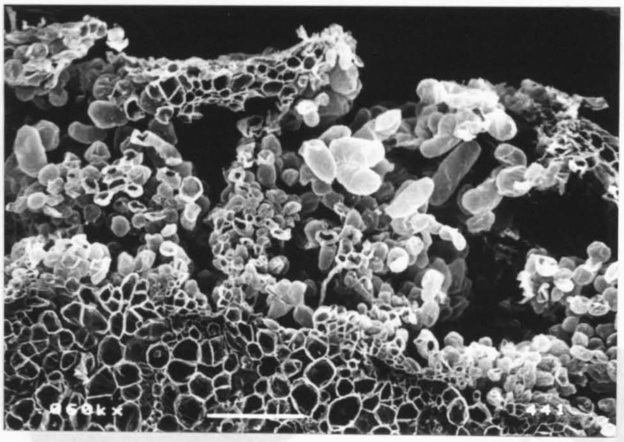

Fig. 25. The breakdown of epidermis observed in plants subjected to continuous waterlogging for 8 days (x60).

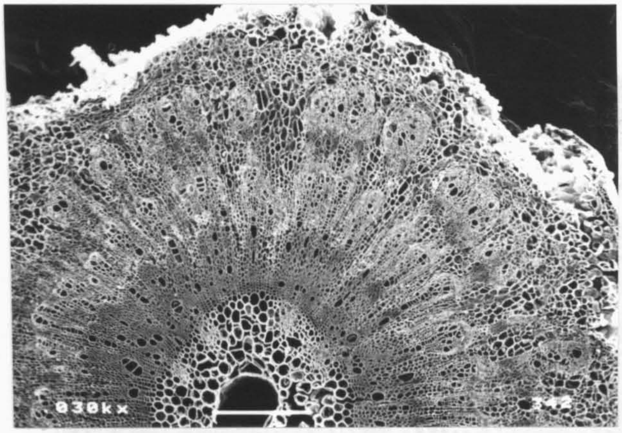

Fig. 26. Transverse section of the basal part of a stem in plants subjected to waterlogging for 4 days on 4 days after the withdrawal of waterlogging (x30).

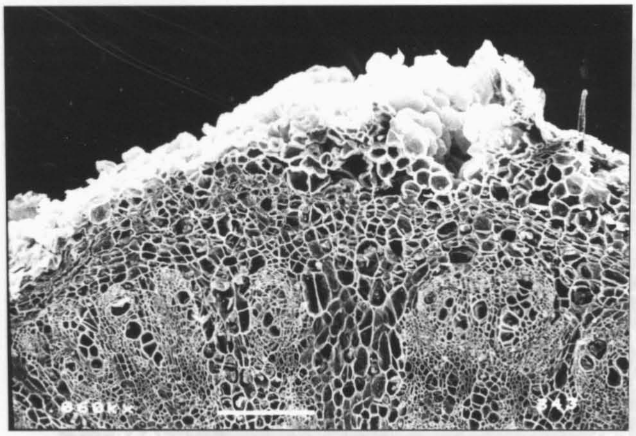

Fig. 27. Transverse section of the basal part of a stem in plants subjected to waterlogging for 4 days on 4 days after the withdrawal of waterlogging (x60).

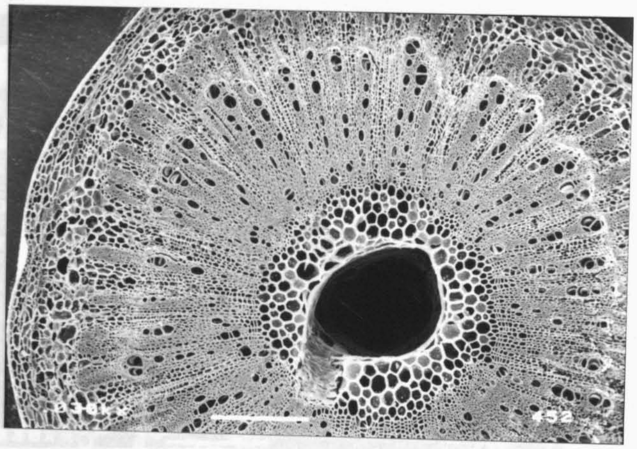

Fig. 28. Transverse section of the basal part of a stem in non-waterlogged plants on 13 days after the onset of the treatment (x30).

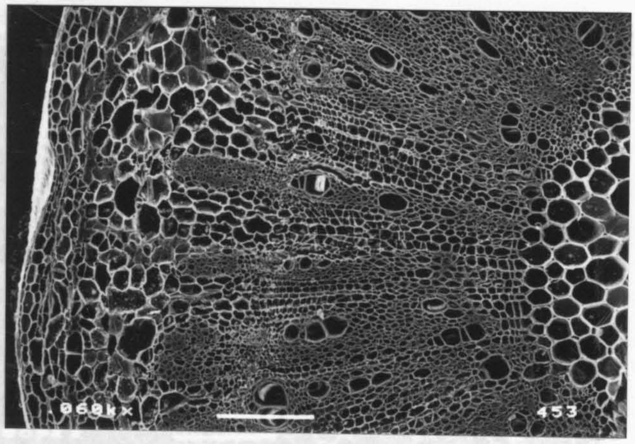

Fig. 29. Transverse section of the basal part of a stem in non-waterlogged plants on 13 days after the onset of the treatment (x60).

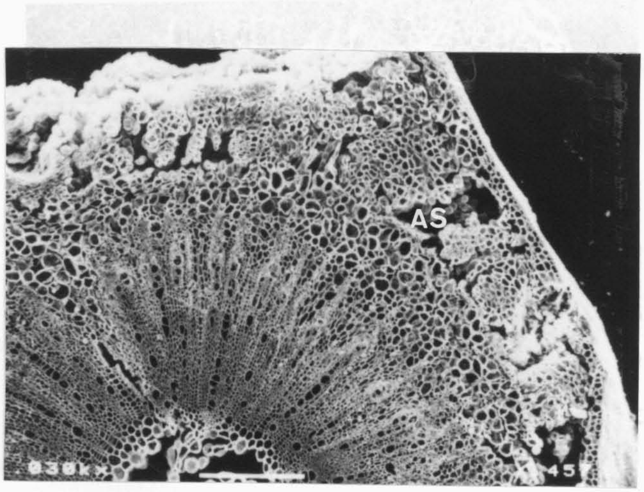

Fig. 30. Transverse section of the basal part of a stem in waterlogged plants on 13 days after the onset of waterlogging (x30). AS; air space.

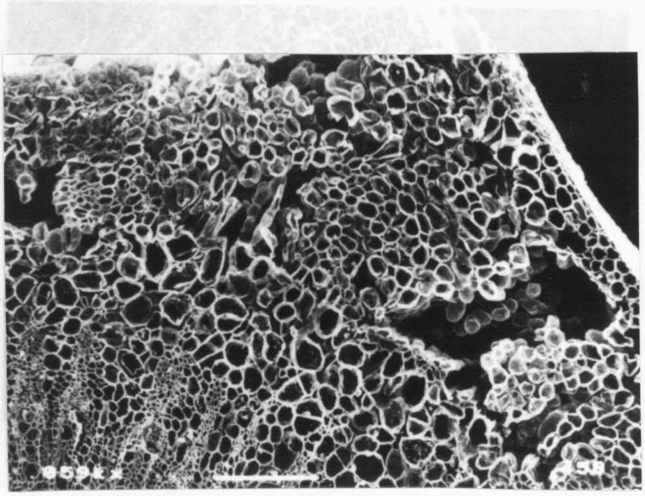

Fig. 31. Transverse section of the basal part of a stem in waterlogged plants on 13 days after the onset of waterlogging (x60).

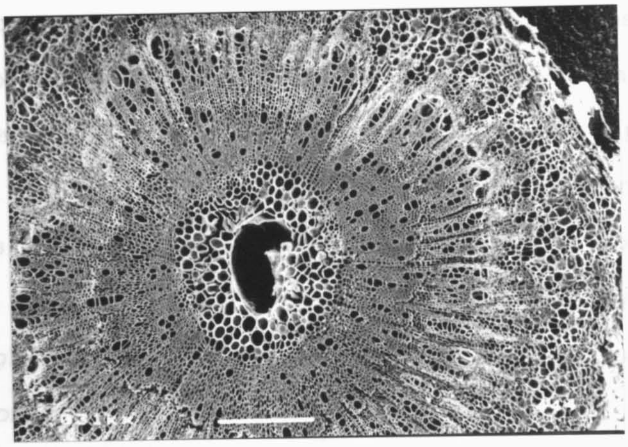

Fig. 32. Transverse section of the basal part of a stem in plants subjected to waterlogging for 4 days on 9 days after the withdrawal of waterlogging (x30).

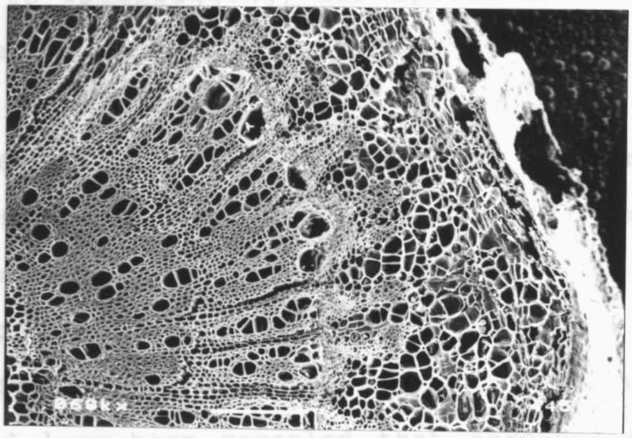

Fig. 33. Transverse section of the basal part of a stem in plants subjected to waterlogging for 4 days on 4 days after the withdrawal of waterlogging (x60).

Discussion

Plants grown in non-waterlogged soils are generally exposed to an oxygen-rich environment over most of their shoot and root surfaces. In these cases only limited longitudinal movement of gases may occur within the plant (Krizek, 1982). On the other hand, in plants grown in waterlogged soils, the mode of gas exchange and transport is very different. Once plants are waterlogged, supply of oxygen from rhizosphere to roots is blocked and root respiration is switched from aerobic to anaerobic, resulting in low energy yield. Hydrophytes such as rice and some kind of marsh plants have internal ventilating systems, which enable those plants to transfer enough oxygen from aerial parts to roots longitudinally (Arikado 1959, 1975; Teal and Kanwisher, 1966; Yamasaki, 1955). If some of mesophytic plants are subjected to waterlogging, air spaces or aerenchyma are formed after the onset of the stress (Arikado, 1975; Bryant, 1934; Drew et al., 1979; Dunn, 1921; Kawase, 1980, 1981; Inden, 1956; McPherson, 1939). Developed aerenchyma under such conditions is considered to function as a pathway of oxygen from the above-ground portion to roots for the improvement of oxygen deficiency of roots.

Observation of hypertrophied stem tissue of waterlogged yard long bean revealed that aerenchyma was formed soon after the beginning of waterlogging. Only 2 days after the onset of the stress, aerenchyma was formed in cortical zone. Existence of rudimental cell walls indicated that air space is of lysigenous origin. As waterlogging period prolonged, such lysigenous aerenchyma became larger. At the same time, epidermis and cortex were destructed and bare cell walls were exposed. Finally white, spongy tissue was formed. As a result of epidermal destruction, some of air spaces were directly connected to outer air near the water table. Thus these air spaces may function both as inlets of oxygen from ambient air to plants and as oxygen pathway from this tissue to roots. As stated before, Arikado (1975) found a similar kind of tissue in a waterlogged wild soybean and called it secondary aerenchyma. He attributed waterlogging tolerance of the wild soybean to the development of secondary aerenchyma and the formation of adventitious roots. In yard long bean, this system also seems to play a large role for oxygen supply to roots. As most of active adventitious roots were formed on this tissue and located near the water surface, distance for oxygen transport may be very short. Effective transport was possible.

Even if waterlogging was withdrawn, aerenchyma development and epidermal and cortical destruction further proceeded for some period, but eventually the hypertrophied tissue was dried and collapsed. Upon the removal of waterlogging, adventitious root formation was retarded in the above-ground part, but that was going on in the soil. Primary roots also recovered their activities after the withdrawal of waterlogging. As the recovery of those root systems and the enrichment of oxygen in rhizosphere, the function of the hypertrophied tissue as secondary aerenchyma seemed to be quickly lost. It is not clear whether the existence of collapsed hypertrophied tissue is disadvantageous to plant growth or not.

Section 2. Physiological changes

Introduction

Vast amount of data was accumulated concerning physiological changes of plants caused by soil waterlogging (Bradford and Yang, 1981; Glinski and Stepniewski, 1985; Krizek, 1981). Well known physiological changes include reduction of root aerobic respiratory activity (Hassan et al., 1986) and a shift to anaerobic respiration (McManmon and Crawford, 1971; Smith and Rees, 1979), accumulation of ethanol in roots resulting from anaerobic respiration (Barta, 1980 ,1984; Bolton and Erickson, 1970; Crawford, 1967), inhibition of hormone biosynthesis in roots such as gibberellins (Reid et al., 1969; Reid and Crozier, 1971) and cytokinins (Burrows and Car, 1969), accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC), a direct precursor of ethylene, in roots and resulting ethylene increase in the above-ground portions (Bradford and Dilley, 1978; Bradford and Yang, 1980, 1981; Jackson and Campbell, 1975; Kawase, 1972, 1974, 1976, 1978), accumulation of auxin in the lower part of stem caused by block of polar transport of auxin to roots (Phillips, 1964), increase of abscisic acid (ABA) in shoots (Jackson et al., 1988; Wadman-van and van Andel, 1985), accumulation of proline mainly in leaf and stem (Wample and Bewley, 1975; Aloni and Rosenshtein, 1982), reduction of water uptake (Kozlowski and Pallardy, 1979; Save and Serrano, 1986; Willey, 1970; Wadman-van and van Andel, 1985), decrease of mineral uptake (Jackson, 1979;

Leyshon and Sheard, 1974) and decline of photosynthetic activity (Pezeshki and Sundstrom, 1988). Just like various morphological changes induced by soil waterlogging, physiological changes also include both damages by and adaptive features to the stress condition.

Most of those physiological changes are considered to indicate the extent of the responses to waterlogging or the tolerance to such conditions in certain species. For example, waterlogging tolerant marsh plants do not accumulate ethanol as much as those intolerant to waterlogging (Crawford, 1967). Proline accumulation in tolerant tomato varieties are much smaller than that in intolerant varieties (Aloni and Rosenshtein, 1982). Generally, it is considered that if those physiological changes are small, the tolerance to waterlogging may be high.

In this section, some of important physiological changes in yard long bean were investigated. In the previous chapter, it was clarified that yard long bean was tolerant to root oxygen deficiency itself. There is a possibility that some of physiological factors are related to the observed tolerance of yard long bean. As in the previous experiments, soils were sterilized before use to analyze the direct effects of root oxygen deficiency on physiological changes under waterlogging conditions.

Materials and methods

Yard long bean, cv. 'TKC-83', were sown on June 6th in 1989 and transplanted 13 days later. Cultural practices and the treatments were the same as those described previously. Appropriate number of plants were subjected to waterlogging for 4 days (short-term treatment) and all the remaining period until the termination of the experiment (continuous treatment). The treatment began at 29 days after sowing when 2-3 trifoliate leaves were expanded.

On 0 (the day when the treatment was started), 2, 4 (the day when short-term treatment was ended), 8 and 13 days (the day when the experiment was terminated) after the beginning of the treatment, the measurement was conducted on the following parameters except for root respiration. Root respiration was measured on 4, 8 and 13 days after the beginning of the treatment. Four replications were used for each parameter.

About 1 g (fresh weight) fine roots were sampled and respiration rate was estimated. Root samples were put in a small plastic box. Two 6 mm diameter plastic tubes, one of them was for an inlet of gas and the other for outlet, were attached to the plastic box. Two plastic tubes were then connected to an air flow pump and a gas analyzer ('ADC LCA2', Shimazu Co. Ltd.). Fresh air was applied to the plastic box at a flow rate of 300 ml/minute and the difference between CO_2 concentration of fresh air and that of air which passed through the plastic box was determined as respiration rate of roots. Remaining roots were used for analyses of ethanol and ACC. For ethanol determination, about 1 g (fresh weight) fine roots were cut into small pieces and extracted in acetone at -20 °C. Ten µl of the extracts were then analyzed with a gas-liquid chromatography ('GC-4CM', Shimazu Co. Ltd.). Conditions for GLC analysis were the same as those described by Lee et al. (1982). ACC analysis was conducted according to Lizada and Yang (1979) using 3 to 6 g (fresh weight) of roots with a little modification. Adsorbed materials to cation exchange resin were eluted by NaOH instead of NH_4OH. In the measurement of ACC contents, sampled fine roots of each replication were combined into one sample, and combined materials were extracted and analyzed. In plants subjected to waterlogging treatments, adventitious root development was observed on 8th and 13th day. Used fine roots for the measurement for those parameters included both primary and adventitious roots.

Diffusive resistance and transpiration rate on the abaxial surface of an apical leaflet in a newly expanded leaf were determined between 1:00 p.m. and 2:00 p.m. by a diffusion porometer ('LI-1600', Licor Co. Ltd.). Water potential of the same leaflet was measured with a pressure chamber ('PC-40', Daiki Rika Co. Ltd.). One of the other leaflets in the same leaf was sampled and stored at -20 °C for analysis of proline accumulation. After all the sampled leaflets were collected proline content was determined according to Bates (1973).

Results

Fig. 34 shows effects of waterlogging treatments on root respiration rate. Four days after the beginning of the treatment, root respiration rate was a little bit higher in waterlogged plants than those in non-waterlogged plants without statistical significance. On 8th day, respiratory activity was increased in plants subjected to both short-term and continuous waterlogging. Thirteen days after the beginning of the treatment, there was still a tendency that plants which experienced short-term waterlogging and continuous waterlogging had higher respiration rates in comparison to non-waterlogged plants.

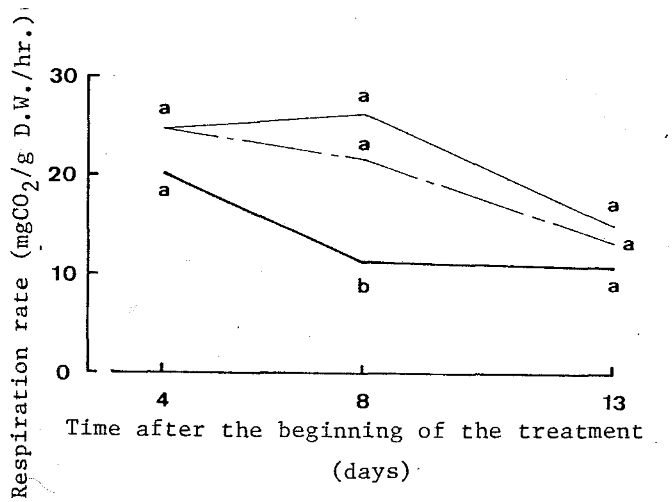

Fig. 34. The effect of waterlogging on root respiration rate. Thick line; non-waterlogged, thin line; subjected to continuous waterlogging, chain line; subjected to short-term waterlogging. Different letters at each measurement time indicate significant difference at p=0.05.

Root ethanol contents in waterlogged plants were higher on 2 and 4 days after the beginning of the treatment (Fig. 35). After the withdrawal of the treatment, ethanol concentrations in roots were lowered on 8th day in short-term waterlogged plants. In plants subjected to continuous

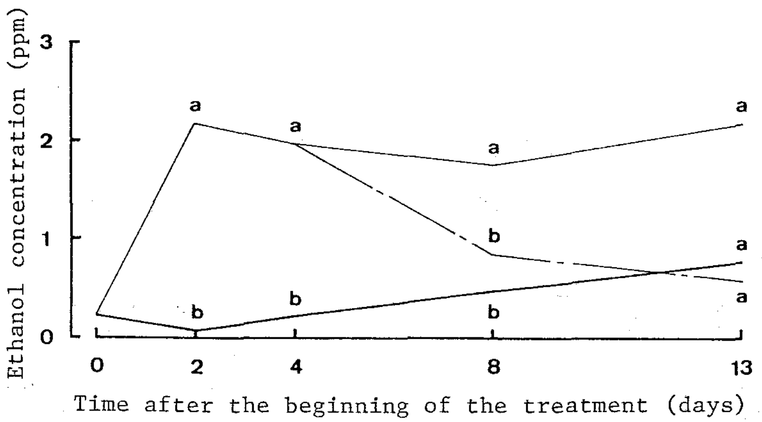

Fig. 35. The effect of waterlogging on root ethanol contents. Thick line; non-waterlogged, thin line; subjected to continuous waterlogging, chain line; subjected to short-term waterlogging. Different letters at each measurement time indicate significant difference at p=0.05.

waterlogging, higher ethanol concentrations were maintained. Thirteen days after the beginning of the treatment, the same tendency was observed.

Root ACC contents in plants subjected to waterlogging were found to be higher than those in non-waterlogged control plants on 2 and 4 days after the onset of waterlogging (Fig. 36). Upon the removal of the treatment, ACC concentrations in roots were lowered on 8 days after the beginning of the treatment. In plants subjected to waterlogging continuously, they remained to be higher. On 13th day, ACC concentrations in roots were lowered to those of control plants in plants subjected to both short-term and continuous waterlogging.

Fig. 37 and Fig. 38 show effects of waterlogging treatments on changes of transpiration rate and diffusive resistance. Two days after the beginning of the treatment, cwaterlogged did no affect both parameters. Four days after

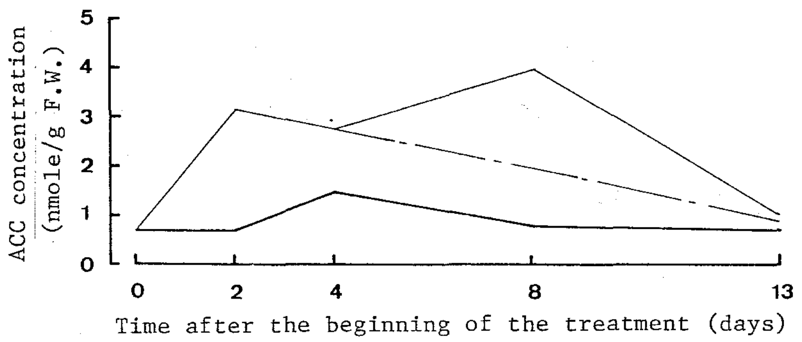

Fig. 36. The effect of waterlogging on root ACC contents. Thick line; non-waterlogged, thin line; subjected to continuous waterlogging, chain line; subjected to short-term waterlogging. As combined samples were analyzed, mean separation could no be done.

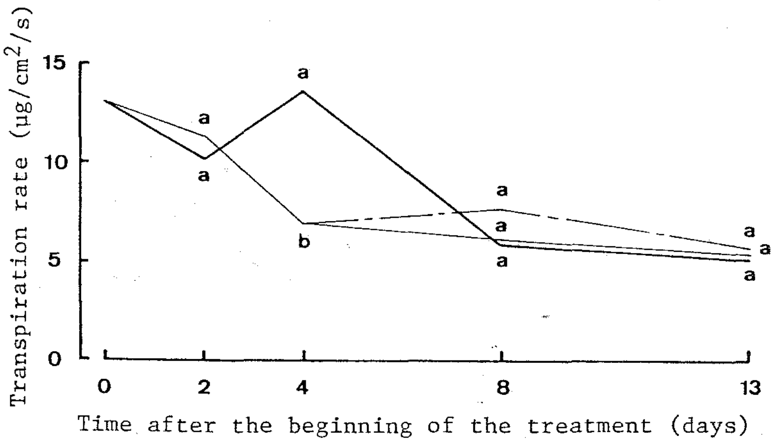

Fig. 37. The effect of waterlogging on leaf transpiration rate. Thick line; non-waterlogged, thin line; subjected to continuous waterlogging, chain line; subjected to short-term waterlogging. Different letters at each measurement time indicate significant difference at p=0.05.

the beginning of the treatment, restriction of transpiration rate and increase of diffusive resistance were observed in waterlogged plants. Thereafter, both transpiration rate and

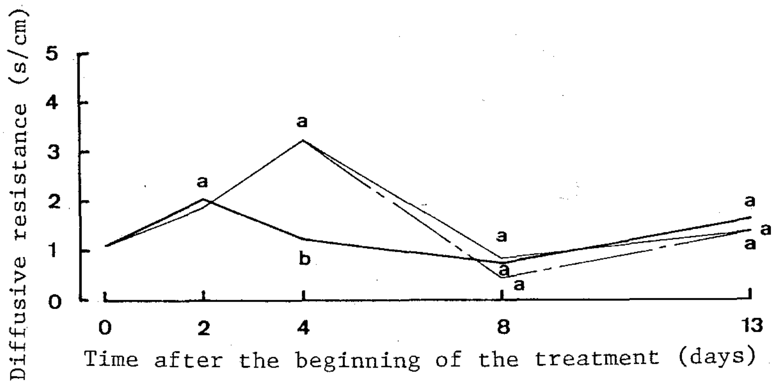

Fig. 38. The effect of waterlogging on leaf diffusive resistance. Thick line; non-waterlogged, thin line; subjected to continuous waterlogging, chain line; subjected to short-term waterlogging. Different letters at each measurement time indicate significant difference at p=0.05.

diffusive resistance were not affected by the treatments. Throughout the experimental period, wilting symptom was not observed in all the treatment plots.

Leaf water potentials were not affected by waterlogging treatments throughout the experiment period (Fig. 39).

Fig. 40 shows effects of the treatments on changes of leaf proline contents. Two days after the beginning of the treatment, proline contents were significantly increased by waterlogging. On 4th day proline contents showed a tendency to increase without statistical significance. Eight days after the beginning of the treatment, proline contents were not influenced by the treatments. On 13th day a little but significant increase was observed in plants waterlogged continuously.

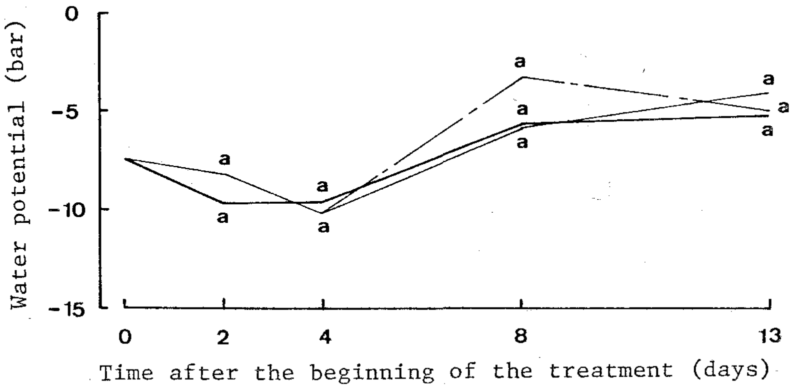

Fig. 39. The effect of waterlogging on leaf water potential. Thick line; non-waterlogged, thin line; subjected to continuous waterlogging, chain line; subjected to short-term waterlogging. Different letters at each measurement time indicate significant difference at p=0.05.

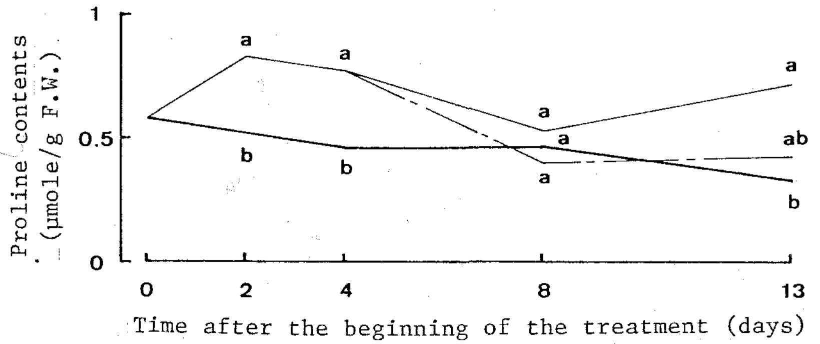

Fig. 40. The effect of waterlogging on leaf proline contents. Thick line; non-waterlogged, thin line; subjected to continuous waterlogging, chain line; subjected to short-term waterlogging. Different letters at each measurement time indicate significant difference at p=0.05.

Discussion

Suppression of root aerobic respiration is commonly recognized as a primary symptom of plants subjected to root oxygen deficiency (Fitter and Hay, 1981; Krizek, 1982).

The result obtained here is rather different from such general agreement. Four days after the onset of waterlogging, root respiratory activity was not reduced by the treatment. As stated in the previous chapter, primary roots were gradually blackened and finally rotted as waterlogging prolonged. This fact seems to indicate that root oxygen deficiency gave detrimental effects on primary root respiration. Thus it is difficult to consider that depression of root aerobic respiration did not occur. In this experiment, the measurement of root respiratory activity was conducted in aerobic condition. If respiratory apparatus in cells of roots was destroyed by root oxygen deficiency, the respiration rate in aerobic conditions must have been reduced either. Several preliminary experiments showed that tetrazolium chloride (TTC) reducing power was not decreased by 1 to 4 days waterlogging treatments in yard long bean. The fact that the respiration rate was not reduced by 4 days of waterlogging and that TTC reducing power was maintained for several days after the onset of waterlogging may reveal that the aerobic respiratory apparatus itself is not affected by the treatment and that if roots are exposed to aerobic conditions, root aerobic respiratory activities will soon recover. The recovering ability of yard long bean from short-term waterlogging may be partly attributed to the maintained root respiratory apparatus during waterlogging for some period.

On 8 and 13 days after the beginning of the treatment, respiratory activities in plants subjected to both the short-term and the continuous treatment were even higher than control plants. At these stages, the adventitious roots were actively formed and hypertrophied stem tissue with developed aerenchyma was nearly established in continuously waterlogged plants. Thus respiratory activities of the adventitious roots may have become high in plants subjected to waterlogging. In plants subjected to short-term waterlogging, removal of waterlogging induced the recovery of primary roots along with adventitious root formation in the soil, presumably resulting in the increase of root respiration rates.

Although root ethanol content of yard long bean was significantly increased by waterlogging at any measurement time, the extent of increment was small in comparison to other species such as tomatoes (Bolton and Erickson, 1970) and alfalfa (Barta, 1980). It is recognized that hydrophytes accumulate much smaller amount of ethanol under waterlogging conditions (Crawford, 1967; McManmon and Crawford, 1971). This phenomenon is generally explained by both ample supply of oxygen from the above-ground portions to roots, which becomes possible by the development of inner ventilating system in those species (Fitter and Hay, 1981), and the alternate metabolic pathway other than glycolysis or alcohol fermentation, which is considered to be the ordinary anaerobic respiratory pathway (McManmon and Crawford, 1971; Fitter and Hay, 1981). On the other hand, in mesophytes, ethanol rapidly accumulates as a result of anaerobic respiration, along with the suppression of transpiration, which is considered to be a major way of ethanol excretion (Jackson et al., 1982). In the case of yard long bean, as described later, the restriction of transpiration was small. Thus accumulated ethanol appeared to be effectively excreted by transpiration until the development of hypertrophied tissue and adventitious roots, and low level of ethanol content in roots was maintained. The ethanol contents found here were much lower than toxic levels (Jackson et al., 1982).

ACC accumulation in roots and consequent ethylene synthesis in shoots after the transportation of ACC to shoots are well known physiological responses of plants to root anaerobiosis (Bradford and Yang, 1980, 1981). In this experiment, elevation of ACC contents in roots was found for some period after the onset of waterlogging. After the removal of waterlogging or the formation of adventitious roots, however, ACC contents in roots were reduced to the level comparable to that in non-waterlogged controls. This shows that ACC accumulation in roots was the direct result of root oxygen deficiency. Some of representative morphological changes caused by waterlogging, such as leaf epinasty, leaf abscission, stem hypertrophy and adventitious root formation were considered to be promoted by ethylene (Bradford and Yang, 1981; Jackson, 1985; Kawase, 1981; Konings and de Wolf, 1984). As those changes include both adaptive changes and simple damages, it seems difficult to relate the degree of the ACC or ethylene elevation to the tolerance to waterlogging.

The fact that leaf water potential was not affected by waterlogging treatments and that no wilting symptom was observed during the experiment suggested that dehydration of leaves, which is sometimes observed under waterlogging conditions in various crops (Aloni and Rosenshtein, 1982; Kozlowski and Pallardy, 1979; Save and Serrano, 1986; Willey, 1970; Wadman-van and van Andel, 1985), was not induced in yard long bean. On the other hand, transpiration rate was restricted and diffusive resistance was increased by waterlogging on 4 days after the beginning of the treatment. This fact suggested that leaf stomata partially closed by waterlogging for 4 days. The stomatal closure without leaf dehydration in plants subjected to waterlogging was also reported in tomato (Jackson et al., 1978) and pea (Jackson and Kowalewska, 1983). This phenomenon was supposed to be related to ABA accumulation in leaves (Jackson et al., 1988), but its adaptive significance has not been clarified. On 2,8 and 13 days after the onset of waterlogging, transpiration rate and diffusive resistance was not affected by the treatment. The result obtained in this experiment seems to suggest that root oxygen deficiency does not induce severe water stress in yard long bean plants.

Significant increase of leaf proline contents was found in waterlogged plants on 2 and 4 days after the onset of waterlogging. This agreed with the results in sunflower (Wample and Bewley, 1975) and in tomato (Aloni and Rosenshtein, 1982). Proline accumulation was observed in plants grown under various stresses, including drought stress

(Aspinall and Paleg, 1981). Aloni and Rosenshtein (1982) suggested that proline accumulation was closely related to leaf dehydration associated with the flooding stress. In this experiment, proline accumulation was observed without leaf dehydration, and accumulated proline contents were much lower than those reported in tomato (Aloni and Rosenshtein, 1982), suggesting that the increase of proline contents could occur independently from leaf water status. This small but significant proline accumulation may be related to partial stomatal closure without dehydration. Further study would be necessary.

The removal of waterlogging lowered leaf proline contents, suggestive of root recovery. If waterlogging was prolonged, leaf proline contents were once reduced on 8 days after the beginning of the treatment, but increased again on 13th day. The reduction on 8th day seemed to be caused by the formation of hypertrophied tissue and adventitious roots. The cause of second increase observed on 13th day was not clear. The restriction of growth of the above-ground parts observed at this stage (see previous chapter) may be related to this phenomenon.

Section 3. Conclusion

In the previous chapter, it was proved that yard long bean was comparatively tolerant to root oxygen deficiency from several agronomical data. In this chapter, morphological and physiological changes caused by root oxygen deficiency were investigated and the results obtained appear to support the conclusion in the previous chapter.

Anatomical observation using a scanning electronic microscope revealed that lysigenous aerenchyma was developed within white and spongy tissue on hypertrophied stem in plants subjected to waterlogging, irrespective of the duration of stress. Some of the aerenchyma was connected directly to the outer air through the destructed epidermis. The formation of the aerenchyma was followed by the development of adventitious roots. The developed aerenchyma may work both as entrances of oxygen from ambient air into plants and as oxygen pathway from the white and spongy tissue to newly developed adventitious roots, which may have functions to absorb water and nutrient vigorously instead of primary roots. As proper supply of oxygen to root system is prerequisite for the adaptation of plants to waterlogging conditions, the formation of such aerenchyma and the development of adventitious roots play an important role for the tolerance of this crop to waterlogging, especially with long-term.

Various physiological changes induced by waterlogging seem to sustain the view that yard long bean is tolerant to root oxygen deficiency. The maintenance of root aerial respiratory apparatus, low level of root ethanol accumulation, the absence of leaf dehydration and low proline accumulation are considered to indicate the relative tolerance of this crop to root oxygen deficiency. Especially the ability to maintain the apparatus for aerobic respiration in primary roots may be important for the post-stress recovery after short-term waterlogging.

The meaning of some of physiological changes was not clarified yet. Those include partial stomatal closure without leaf dehydration and small but significant increase of leaf proline. Further studies are required.

Chapter 4. Varietal differences in plant responses to waterlogging

Section 1. Varietal differences in responses to short-term waterlogging

Introduction

In the previous 2 chapters, various factors which influence responses of yard long bean plants to waterlogging, and morphological and physiological changes caused by root oxygen deficiency were discussed using one certain variety. In this chapter, varietal differences in responses to waterlogging are examined, partly to confirm the results obtained in the former chapters using other varieties, and mainly to acquire fundamental information for breeding for waterlogging tolerance. In this section, the effects of varietal differences on responses to short-term waterlogging at different growth stages are investigated. In this study also, soils were sterilized before use to minimize effects of other factors than root oxygen deficiency.

Materials and methods

Yard long bean plants, cv. 'TKC-83', 'I-2939-Wonosobo' (Indonesian variety), 'Akadane-Sanjaku' (Japanese variety) and 'Kurodane-Sanjaku' (Japanese variety) were sown on May 11th in 1988 and transplanted 10 days later. Cultural practices and the treatments were the same as those described in the section 1 in Chapter 2. Fluctuation of

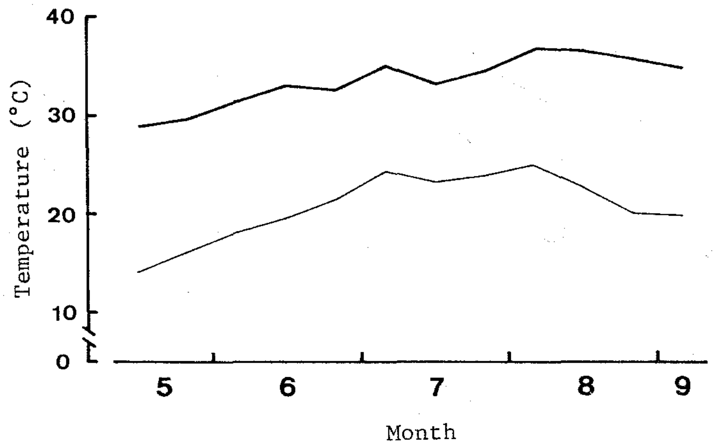

Fig. 41. The fluctuation of ambient maximum (-) and minimum (-) temperature during the experiment.

ambient temperature during this experiment is shown in Fig. 41. Appropriate number of plants were subjected to waterlogging for 4 days at the vegetative stage (2-3 trifoliate leaves expanded) and at the flowering stage (first flowers just opened).

The experiment was conducted as a randomized block design with 8 replications in a factorial arrangement of 4 varieties and 3 waterlogging treatments including non-waterlogged control. Plants were spaced 30 cm in-row and 70 cm between-row.

Seed yield and several characteristics relating to yield were determined. Additionally, the number of leaves, length of 3 to 6 developing leaves and chlorophyll contents of several fully matured leaves were measured at appropriate intervals during and after the treatments in order to estimate the effects of waterlogging on growth. Leaf chlorophyll contents were measured with a Green meter (Fuji Film Co. Ltd.).

Results

Fig. 42 shows the varietal differences in the effects of short-term waterlogging at the vegetative stage on the changes of the number of leaves. In all varieties, the treatment did not affect the changes of the number of leaves.

Chlorophyll contents of matured leaves were affected by short-term waterlogging differently among varie-

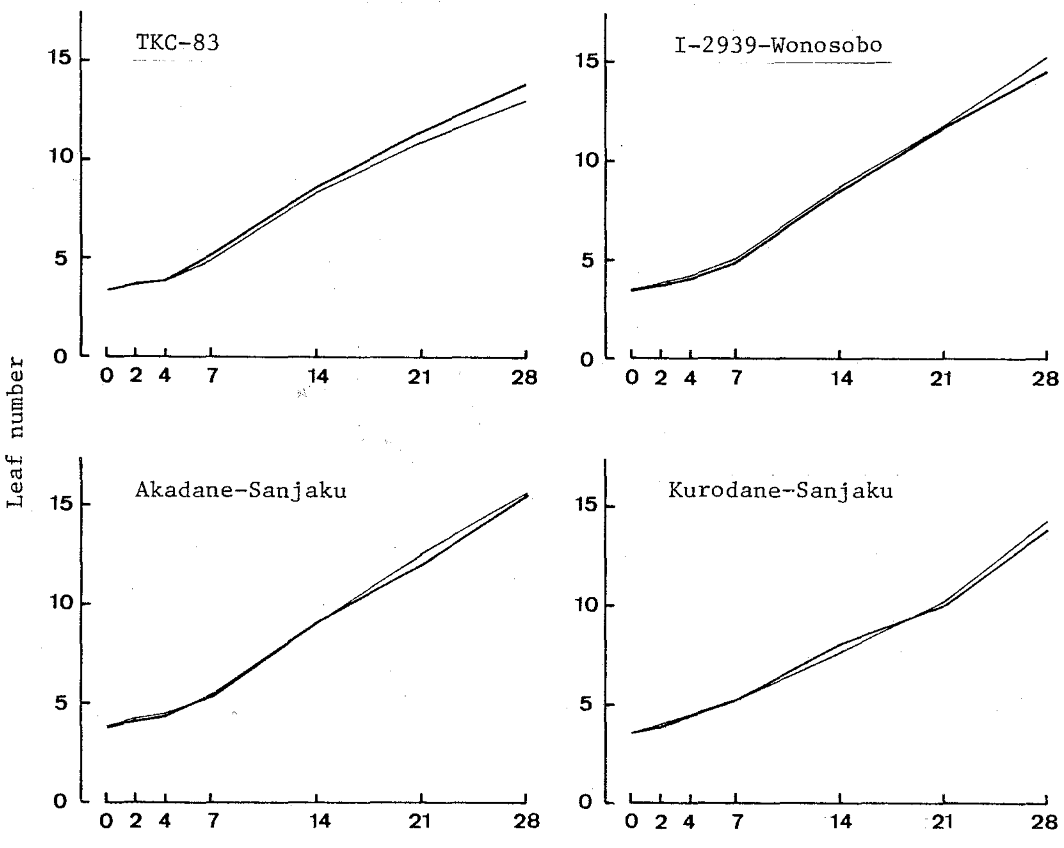

Fig. 42. The effect of short-term waterlogging on the changes in leaf number in 4 varieties of yard long bean. Thick line; non-waterlogged, thin line; waterlogged. Significant difference was not detected at all measurement time in all varieties.

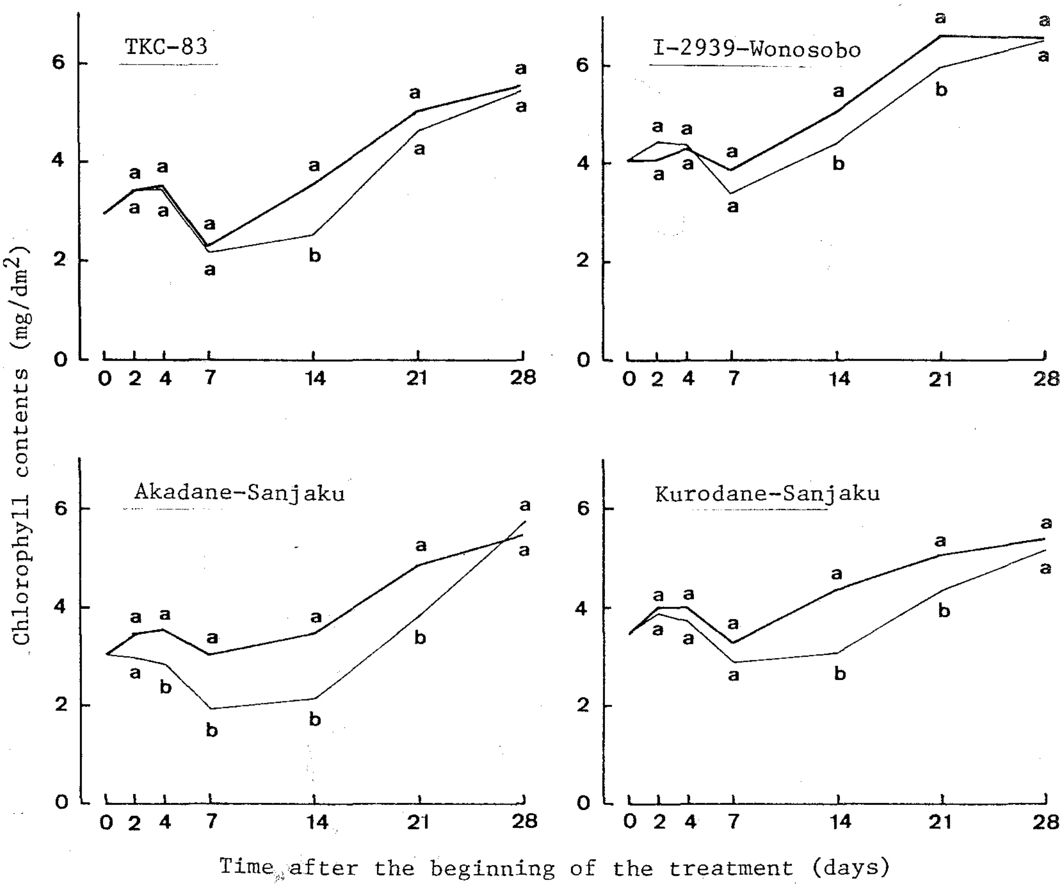

Fig. 43. The effect of short-term waterlogging on the changes in leaf chlorophyll contents in 4 varieties of yard long bean. Thick line; non-waterlogged, thin line; waterlogged. Different letters at each measurement time indicate significant difference at p=0.05.

ties (Fig. 43). In TKC-83, the treatment did not affect leaf chlorophyll contents until 3 days after the withdrawal of the treatment (7 day after the beginning of the treatment). Ten days after the removal of waterlogging (14 days after the beginning of the treatment) chlorophyll contents were significantly reduced, and thereafter recovered to almost the same level as non-waterlogged control. In 'I-2939-Wonosobo', a similar tendency was observed, although recovery in chlorophyll contents was delayed. 'Akadane-Sanjaku' was most severely affected by the treatment. The reduction of leaf chlorophyll contents was found at the time of the removal of the treatment. This variety showed the decrease in chlorophyll contents after the withdrawal of waterlogging, and finally recovered on 21 days after the removal of the treatment. 'Kurodane-Sanjaku' showed the same trend as 'I-2939-Wonosobo'.

Leaf length was also affected by short-term waterlogging variously among varieties (Fig. 44). 'TKC-83' was little affected by the treatment in this parameter. In all leaves, slight reduction without statistical significance was observed. In 'I-2939-Wonosobo', length of the 6th and 7th leaf was reduced significantly. Other leaves did not receive the significant effect of short-term waterlogging. 'Akadane-Sanjaku' was most severely affected by the treatment in this parameter. The reduction of length caused by waterlogging was observed in the leaves upper than the 3rd leaf. 'Kurodane-Sanjaku' was also affected by short-term waterlogging in this measurement item. Leaves on 4th to 7th node showed the significant decrease of their length.

The number of days to first flower was not affected by the waterlogging treatments in all varieties (Table 10).

Fig. 45 shows the varietal differences in the effects of short-term waterlogging at the vegetative and the flowering stage on seed yield. The yield of 'TKC-83' and 'I-2939-Wonosobo' was little affected by the treatments at

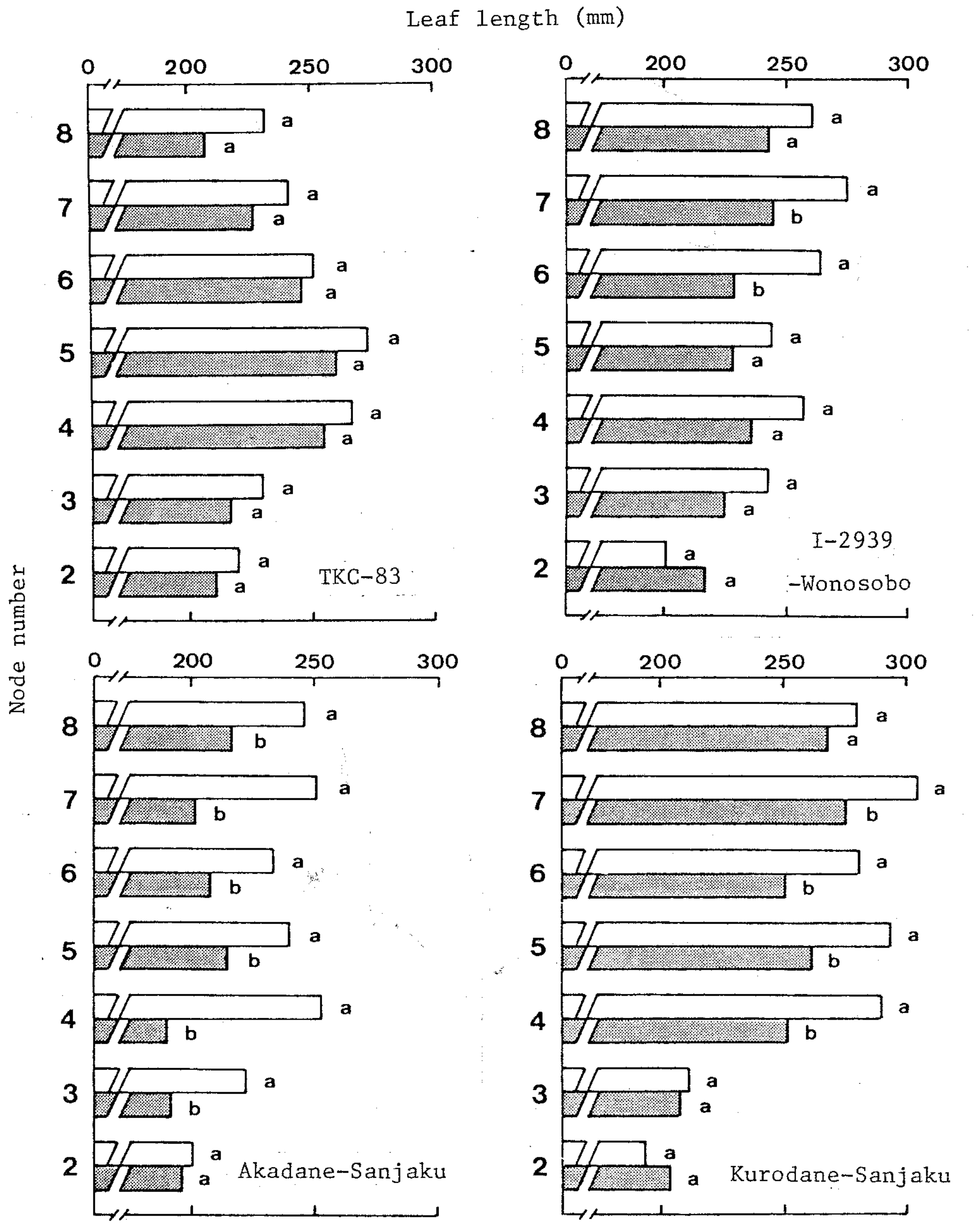

Fig. 44. The effect of short-term waterlogging on the changes in leaf length in 4 varieties of yard long bean. Open bar indicates non-waterlogged plants and dotted bar waterlogged ones. Bars with the same letter do not differ at the 5 % level.

Table 10. The effect of short-term waterlogging on the nuber of days to first flower in several varieties in yard long bean.

Treatment	Number of days to flower Variety TKC-83	 I-2939-Wonosobo	 Akadane-Sanjaku	 Kurodane-Sanjaku
N.W.[1)]	54.2 a[2)]	61.0 a	65.3 a	62.8 a
W.V.	53.5 a	60.7 a	64.8 a	65.2 a
W.F.	52.0 a	61.0 a	65.0 a	64.7 a

1) N.W.; Non-waterlogged.
W.V.; Waterlogged at the vegetative stage.
W.F.; Waterlogged at the flowering stage.
2) Different letters indicate significant difference at p=0.05.

both stages. Waterlogging at the vegetative stage reduced the yield of 'Akadane-Sanjaku' significantly, while the treatment at the flowering stage exerted a limited influence. On the contrary, the yield of 'Kurodane-Sanjaku' was little affected by the treatment at the vegetative stage, and significantly reduced by that at the flowering stage.

Table 11 shows the varietal differences in the effects of short-term waterlogging at both stages on various yield components. In 'TKC-83', the number of pod on main stem in waterlogged plants at the vegetative stage was significantly lower than that in plants subjected to waterlogging at the flowering stage. Non-waterlogged control showed the medium value between plants waterlogged at the vegetative stage and those at the flowering stage without significant differences. In the other parameters, significant effects were not observed by the waterlogging treat-

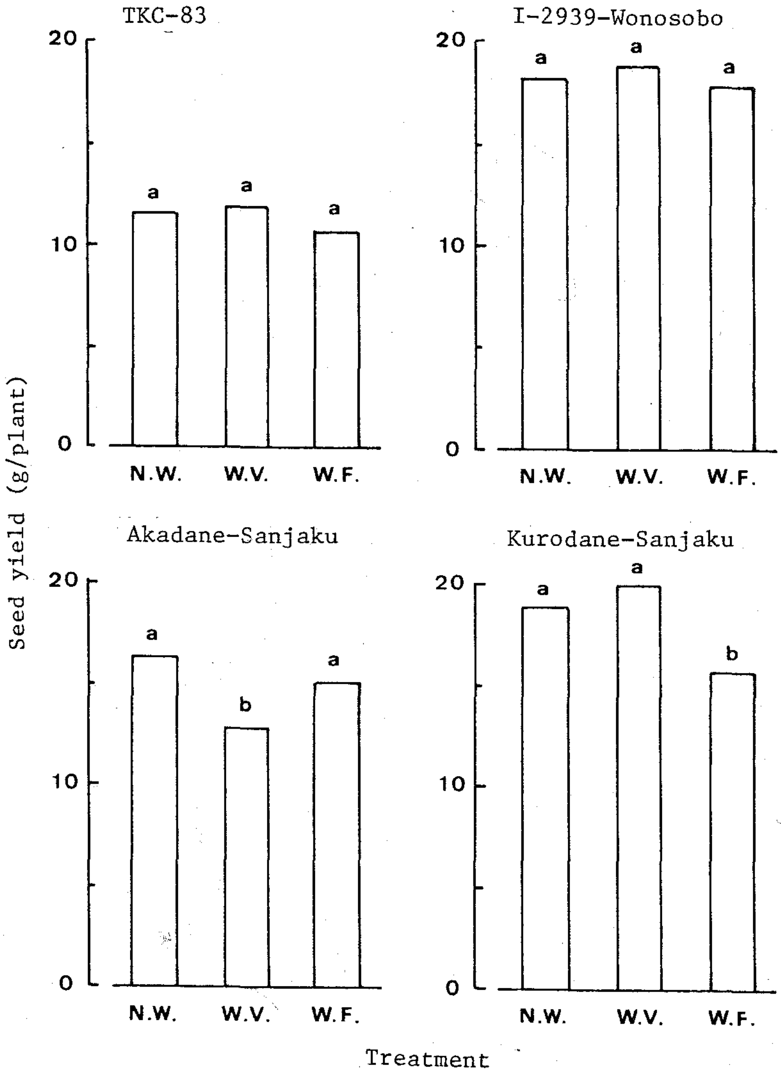

Fig. 45. The effect of short-term waterlogging on seed yield in 4 varieties of yard long bean. Abbreviations are the same as those in Table 10. Columns covered by the same letter do not differ at the 5 % level.

ments. In 'I-2939-Wonosobo', only 100-seed weight was affected by the treatments. One hundred seed weight was not influenced by waterlogging at the vegetative stage, while increased by the treatment at the flowering stages. In 'Akadane-Sanjaku', the number of pod on lateral shoot was decreased significantly by waterlogging at the vegetative stage. The treatment at the flowering stage showed a tendency to reduce this parameter. The other parameters were not affected significantly by the waterlogging treatments. On the contrary to 'Akadane-Sanjaku', 'Kurodane-Sanjaku' showed the reduction in the number of pod on lateral shoot by waterlogging at the flowering stage, while it did not show the effects of the treatment at vegetative stage.

Table 11. The effect of short-term waterlogging on various characteristics relating to yield in several varieties in yard long bean.

Varieties and Treatment	Number of pods Main stem	Number of pods Lateral shoot	Number of seeds per pod	100-seed weight (g)	Pod set rate (%)
TKC-83					
N.W.[1]	8.3 ab[2]	2.7 a	10.2 a	8.9 a	45.7 a
W.V.	7.5 b	3.2 a	10.7 a	9.6 a	49.5 a
W.F.	9.8 a	1.9 a	8.8 a	9.0 a	54.9 a
I-2939-Wonosobo					
N.W.	5.8 a	7.7 a	11.3 a	11.6 b	35.0 a
W.V.	4.8 a	9.0 a	11.0 a	12.0 ab	42.6 a
W.F.	4.5 a	7.8 a	11.3 a	13.9 a	39.9 a
Akadane-Sanjaku					
N.W.	7.0 a	9.8 a	9.7 a	9.3 a	57.6 a
W.V.	6.8 a	5.5 b	10.7 a	9.4 a	48.1 a
W.F.	5.8 a	7.0 a	11.4 a	12.0 a	59.5 a
Kurodane-Sanjaku					
N.W.	6.5 a	10.5 a	9.6 a	10.6 b	57.5 a
W.V.	6.7 a	11.8 a	9.7 a	10.5 b	64.9 a
W.F.	5.8 a	5.4 b	9.9 a	12.3 a	48.7 a

1) Abbreviations are the same as those in Table 10.
2) Different letters indicate significant difference at p=0.05.

Discussion

It has been reported that there are intraspecific differences in the plant response to waterlogging (Krizek, 1982). The current results seem to support this view.

Various parameters concerning the vegetative growth showed the varietal differences in the response to short-term waterlogging except the changes of leaf number. The results on the changes of chlorophyll contents and the leaf length appear to indicate that damages caused by short-term waterlogging were lightest in 'TKC-83', most severe in 'Akadane-Sanjaku' and intermediate in the other varieties.

Seed yield was also affected by short-term waterlogging differently among varieties. In 'TKC-83' and 'I-2939-Wonosobo', the seed yield was not affected significantly by the treatment at both stages. In 'TKC-83', the least damages of vegetative growth may account for the absence of yield reduction by short-term waterlogging at the vegetative stage, although this was not in agreement with the results obtained in the section 1 in Chapter 2. As discussed previously, slight changes of environmental conditions may have induced the inconsistency. The treatment at the flowering stage did not affect the seed yield like the previous experiment. In 'I-2939-Wonosobo', though the damages caused by short-term waterlogging at the vegetative stage were larger than those observed in 'TKC-83', the seed yield was not reduced significantly. The recovering ability may be excellent in this variety. The treatment at the flowering stage did not affect the seed yield either, but it increased 100 seed weight significantly. The cause for this phenomenon was not clear. Waterlogging may have affected the process of seed development in pod.

In 'Akadane-Sanjaku', the seed yield was reduced by waterlogging at the vegetative stage. In this variety, the extent of retardation of the vegetative growth was most among used varieties. This may have induced low yield. Pod number on lateral shoots was also decreased by the treatment and this seems to be related to the yield reduction. The seed yield was not affected by short-term waterlogging at the flowering stage.

In 'Kurodane-Sanjaku', the yield reduction was not observed in plants subjected to short-term waterlogging at the vegetative stage. As the plants showed the damages caused by the treatment to some extent during the vegetative stage, it is considered that they recovered quickly from such damages, resulting in the comparable yield to that of control plants. Short-term waterlogging at the flowering stage, however, reduced the seed yield in this variety. This seems to be closely related to the decrease of pod number on lateral shoots. As pod set rate was not affected by the treatment, the abscission of flower did not increase by the treatment. Considering the fact that flowers on lateral shoots opened later than those on main stem, the treatment at the flowering stage may have affected the flower development on lateral shoot adversely.

The results obtained here confirm that the sensitivity of the plants to waterlogging differed depending on the growth stages. They also indicate that the stage at which plants are most susceptible to the stress differed among varieties. It is also suggested that there are varietal differences in the extent to which short-term waterlogging may affect plants.

Although some varieties used in this experiment showed the reduction of the growth and the yield, the degree of the influence of short-term waterlogging seems to be much less in comparison to the reported results (Tomooka, 1982; Wien et al., 1979). This fact appears to indicate the relative tolerance of this crop to short-term root oxygen deficiency.

Section 2. Varietal differences caused by continuous waterlogging

Introduction

In the previous section, varietal differences in the response of yard long bean to short-term waterlogging were investigated. In this section, the following experiment was carried out to analyze the varietal differences in the response of yard long bean to continuous waterlogging and its ability to adapt to the stress condition. As the previous experiments, soils were sterilized before use to analyze direct effects of root oxygen deficiency on plant growth.

Materials and Methods

Four yard long bean varieties, 'TKC-83', 'I-2939-Wonosobo', 'Akadane-Sanjaku' and 'Kurodane-Sanjaku' were sown on June 21th in 1989 and transplanted 13 days later. Cultural practices and the treatments were the same as those described previously. Changes in air temperature during this experiment are shown in Fig. 46. Appropriate number of plants were subjected to waterlogging for all the remaining period until the termination of experiment, on 29 days after sowing, when 2-3 trifoliate leaves were expanded.

The experiment was conducted as a randomized block design with 5 replications in a factorial arrangement of 4 varieties and 2 waterlogging treatments, i.e. non-waterlogged control and continuous waterlogging. Plants were

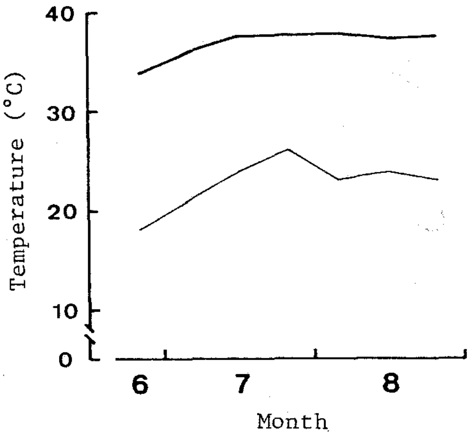

Fig. 46. The fluctuation of ambient maximum (-) and minimum (-) temperature during the experiment.

spaced 30 cm in-row and 70 cm between-row.

The number of leaves, length of 3 to 6 developing leaves and chlorophyll contents of several fully matured leaves were measured at appropriate intervals during the treatments in order to estimate the effects of waterlogging on the growth. Leaf chlorophyll contents were measured with a Green meter (Fuji Film Co. Ltd.). At the same time, morphological changes were also carefully observed. Among such morphological changes, the degree of adventitious root formation was evaluated visually according to the following rate; 0: no adventitious roots observed, 1: adventitious root primordia observed, 2: a few adventitious roots (1-10, short) observed, 3: some adventitious roots (11-20, medium to long) observed, 4: numerous adventitious roots (more than 20, long) observed. Twenty eight days after the onset of the treatment, the experiment was terminated. All the plants were separated into their various components and fresh and dry weights were determined.

Results

In all varieties, waterlogged plants formed white and spongy tissue soon after the onset of waterlogging. Such tissue further developed, and adventitious roots were formed on this tissue several days after the beginning of the treatment. The degree and rate of adventitious root formation caused by waterlogging differed among varieties (Fig. 47). 'TKC-83' initiated adventitious roots later than the other varieties, but developed vigorously afterward. In 'I-2939-Wonosobo', adventitious roots started to be formed 4 to 6 days after the beginning of the treatment, and de-

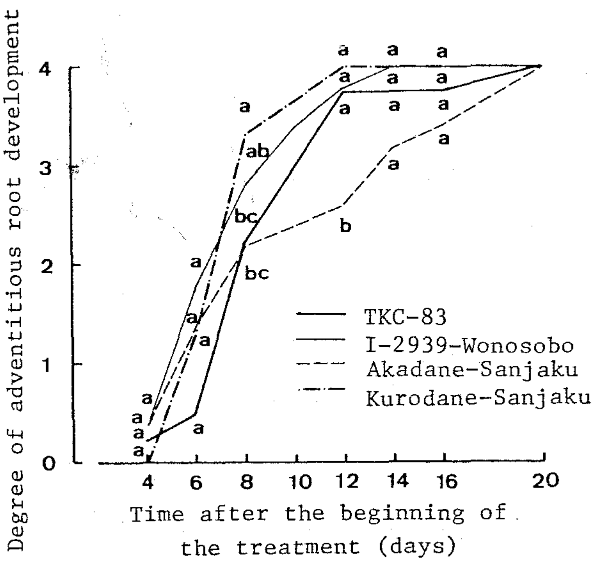

Fig. 47. Varietal difference on the degree of adventitious root development. 0; no adventitious roots (av) observed, 1; primordium of av observed, 2; a few (less than 5) av observed, 3; several av (5 to 20) observed, 4; vigorous av (more than 20) observed. Different letters at each measurement time indicate significant difference at p=0.05.

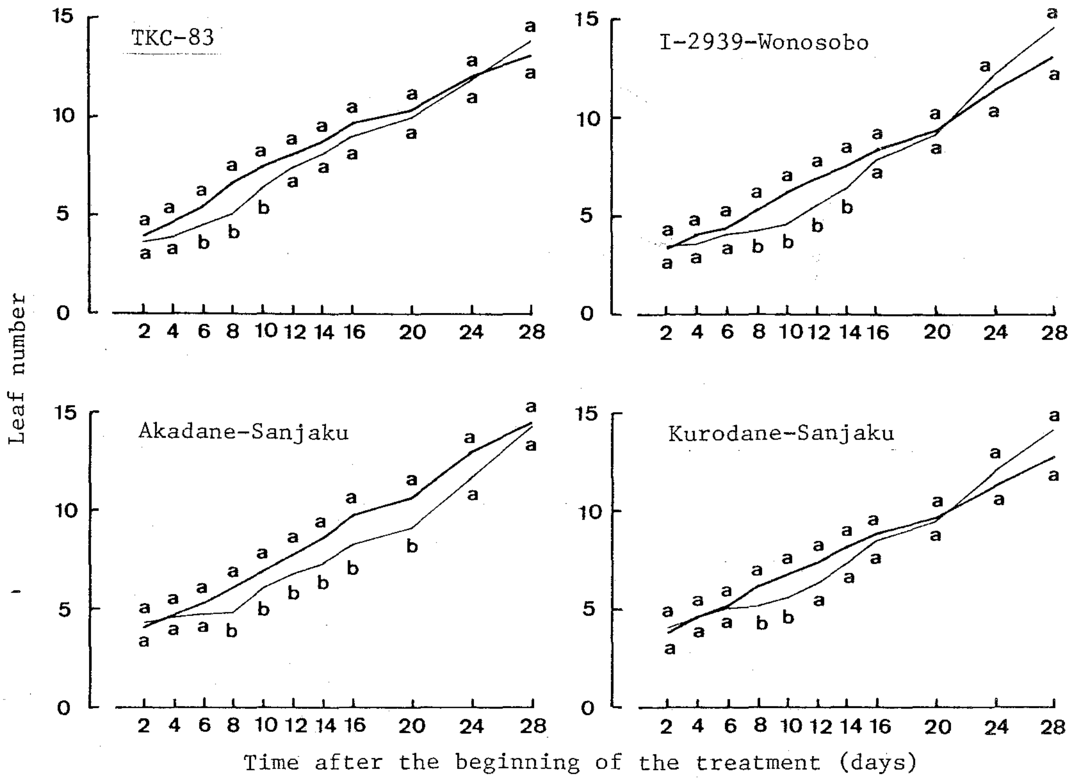

Fig. 48. The effect of continuous waterlogging on the changes in leaf number in 4 varieties of yard long bean. Thick line; non-waterlogged, thin line; waterlogged. Different letters at each measurement time indicate significant difference at p=0.05.

veloped steadily thereafter. 'Akadane-Sanjaku' initiated adventitious roots at about the same time as 'I-2939-Wonosobo' and 'Kurodane-Sanjaku', but rate of development was slower than the other varieties. 'Kurodane-Sanjaku' showed the similar trend as 'I-2939-Wonosobo'.

Fig. 48 shows the effects of continuous waterlogging on the changes in leaf number in each variety. In 'TKC-83', the decrease of leaf number was observed on 6 days after the beginning of the treatment. This tendency continued until 10 days after the onset of waterlogging. Thereafincrease in leaf number. 'I-2939-Wonosobo' received stronger influence by continuous waterlogging. The significant restriction of leaf number was observed on 8 days through 14 days after the beginning of the treatment. 'Akadane-Sanjaku' was affected most severely by continuous waterlogging. Reduction of leaf number was first observed on 8 days after the onset of waterlogging and continued up to 20th day. In 'Kurodane-Sanjaku', significant suppression of leaf number was only found on 8 and 10 days after the beginning of the treatment.

Chlorophyll contents of matured leaves were affected by continuous waterlogging differently among varieties (Fig. 49). In all varieties, the chlorophyll contents in control plants decreased gradually until 16 days after the beginning of the treatment, and increased afterward. In 'TKC-83', the reduction of chlorophyll contents by continuous waterlogging was only observed on 6 days after the beginning of the treatment. The increase of chlorophyll contents was more rapid in waterlogged plants than in non-waterlogged control plants. In 'I-2939-Wonosobo' the effect of waterlogging on the chlorophyll contents appeared even 2 days after the beginning of the treatment, and continued until 10th day. Thereafter, the chlorophyll contents were increased and showed higher value in waterlogged plants than control plants. 'Akadane-Sanjaku' showed a similar tendency to that observed in 'I-2939-Wonosobo', although the recovery of the chlorophyll contents began earlier. In 'Kurodane-Sanjaku', the restriction of the chlorophyll contents by

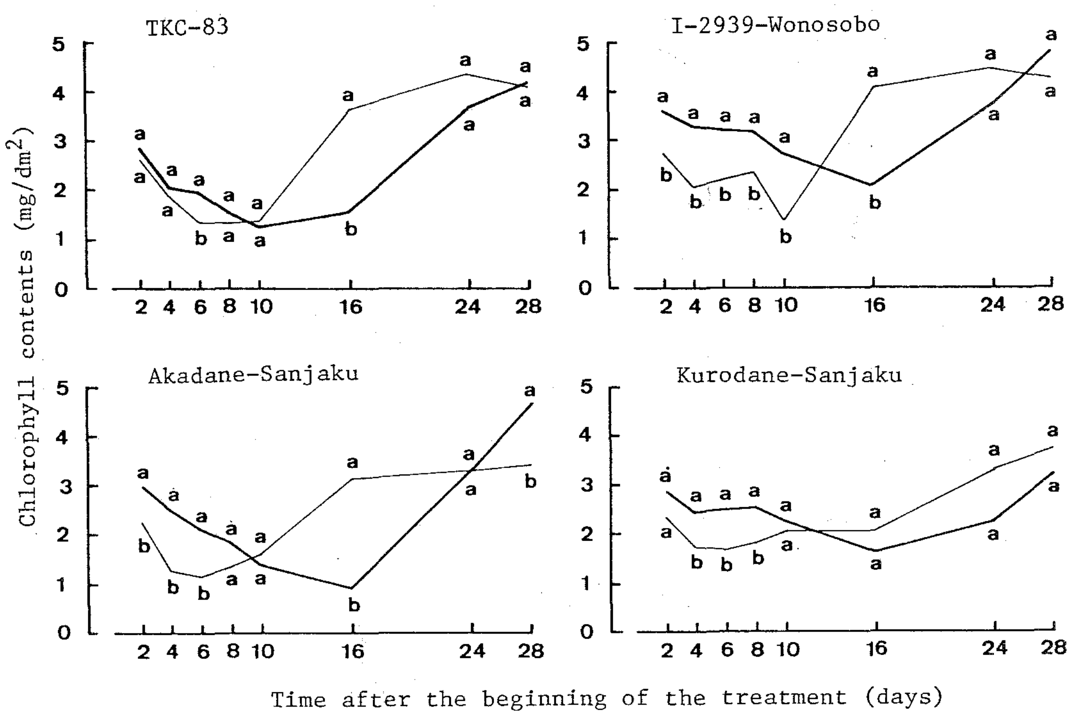

Fig. 49. The effect of continuous waterlogging on the changes in leaf chlorophyll contents in 4 varieties of yard long bean. Thick line; non-waterlogged, thin line; waterlogged. Different letters at each measurement time indicate significant difference at p=0.05.

waterlogging was found on 4 days through 8 days after the beginning of the treatment. After 16th day, the chlorophyll contents in waterlogged plants increased gradually.

Fig. 50 showed the effects of continuous waterlogging on the leaf length and their differences among varieties. In 'TKC-83', length of 4th through 6th leaves was reduced significantly by continuous waterlogging. Leaves on higher than 9th node were longer in waterlogged plants than in non-waterlogged plants. 'I-2939-Wonosobo' showed the restriction of the length in 3rd through 5th leaves. Length

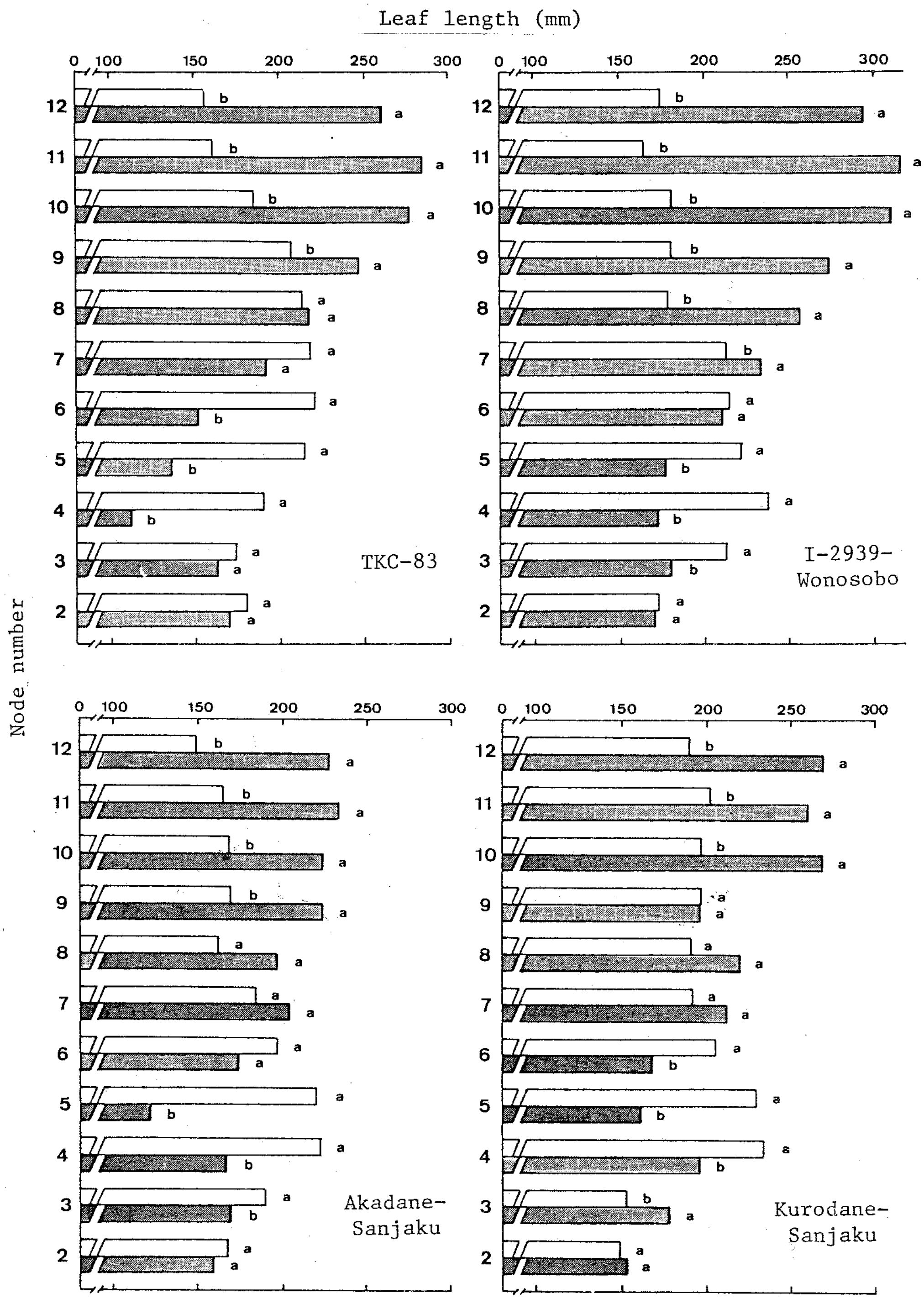

Fig. 50. The effect of continuous waterlogging on the changes in leaf length in 4 varieties of yard long bean. Open bar indicates non-waterlogged plants and dotted bar waterlogged ones. Bars with the same letter do not differ at the 5 % level.

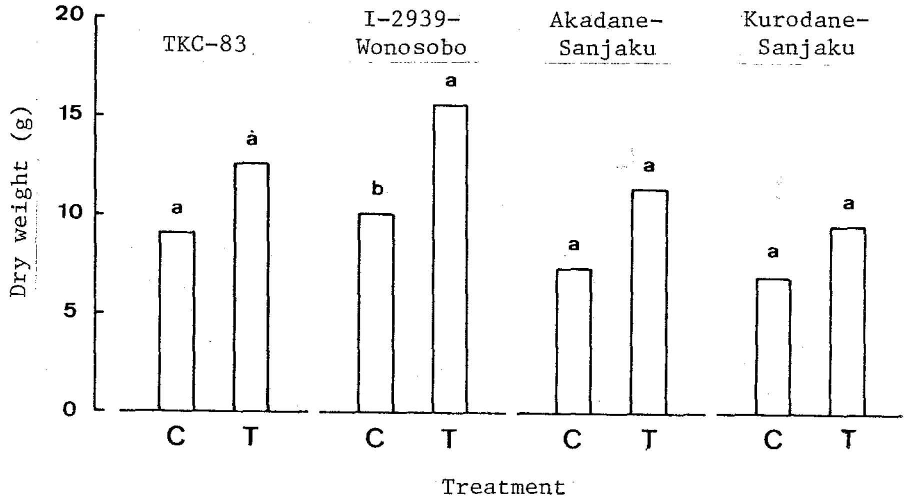

Fig. 51. The effect of continuous waterlogging on dry weight of above-ground parts in 4 varieties of yard long bean on 28 days after the onset of waterlogging. C; control, T; treated. Columns covered by the same letter do not differ at the 5 % level.

of leaves on upper than 6th node became comparable to or even longer than that in control plants. 'Akadane-Sanjaku' and 'Kurodane-Sanjaku' showed the same tendency as that observed in 'I-2939-Wonosobo'.

In all varieties, dry weights of the above-ground part and roots were increased in waterlogged plants than in control plants with or without statistical significance (Fig. 51 and Fig.52).

Discussion

As in the responses to short-term waterlogging, varietal differences were found in the responses to continuous waterlogging on the growth of yard long bean.

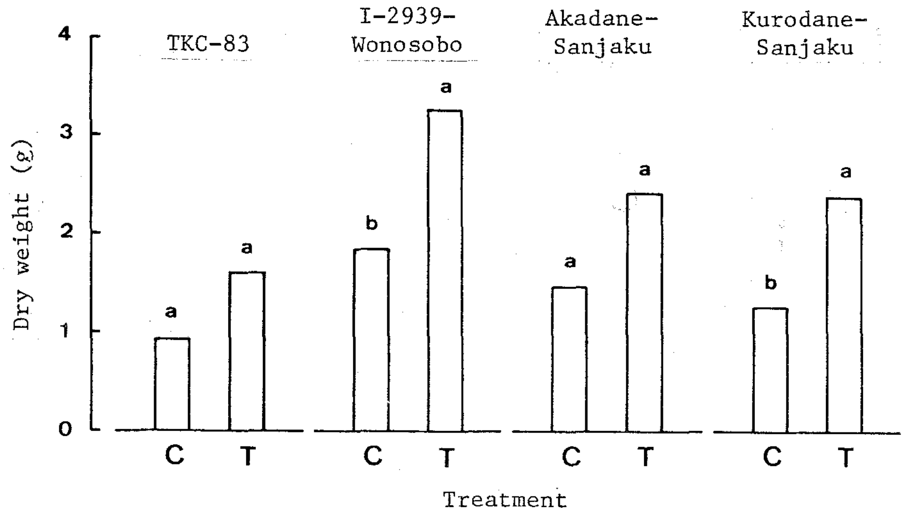

Fig. 52. The effect of continuous waterlogging on dry weight of roots in 4 varieties of yard long bean on 28 days after the onset of waterlogging. C; control, T; treated. Columns covered by the same letter do not differ at the 5 % level.

The rate and degree of adventitious root formation induced by waterlogging was different among varieties. As adventitious root development is essential for the adaptation to continuous waterlogging, this difference may have important meanings.

The results on the changes of leaf number and chlorophyll contents indicated that the extent of waterlogging damages was smaller in 'TKC-83' and 'Kurodane-Sanjaku', and bigger in 'I-2939-Wonosobo' and 'Akadane-Sanjaku'. Slow development of adventitious roots in 'Akadane-Sanjaku' appears to be associated with delayed adaptation to the stress. Although 'I-2939-Wonosobo' received bigger damages by waterlogging, it showed quick and complete recovery, which was indicated in vigorous growth of upper leaves and final dry weight of the above-ground portion and roots.

In this experiment, non-waterlogged plants showed very poor growth in all varieties, as shown in the leaf chlorophyll contents and the final dry weight. The cause was not clear, but considering the fact that non-waterlogged plants showed the recovery from the restricted growth at the end of the experiment, some soil factors may have affected plant growth adversely and leaching out of such factors may have resulted in the recovery of the growth.

Though some differences among varieties were found in the response to continuous waterlogging, all varieties could be adapted to the stress and resume vigorous growth after the adaptation. This fact seems to prove the relative tolerance of this crop to continuous waterlogging. The formation of white and spongy tissue and vital development of adventitious roots may explain the tolerance of yard long bean to root oxygen deficiency.

Section 3. Conclusion

Yard long bean showed the varietal differences in the responses to both short-term and continuous waterlogging.

To short-term waterlogging, some of the varieties showed the susceptibility at the vegetative stage, and some showed at the flowering stage. Mechanisms for the susceptibility were not fully elucidated, but the restriction of the vegetative growth after the withdrawal of waterlogging in the former case, and the suppression of flower development on lateral shoots appear to be related to the susceptibility. The difference of the stage at which plants are most susceptible to the stress, however, may complicate the procedure of the selection for the tolerance to short-term waterlogging, because the selection at both stages must be needed to analyze the tolerance of a given variety.

To continuous waterlogging, yard long bean showed the varietal differences in the vegetative growth. The rate and extent of the development of adventitious roots, which are considered to play a large role in the adaptation to the stress, differed among varieties. This difference may be associated with the difference in the restriction and the recovery of the growth among varieties.

Even though some varieties showed the susceptibility to short-term or continuous waterlogging, severe damages such as abscission of most of leaves and/or death of whole plant did not occur in the experiments in this chapter. All the varieties showed the recovery after the removal of short-term waterlogging, although the degree of the recovery was different among varieties, and adapted themselves to continuous waterlogging with the varietal differences in the rate of adaptation. These facts seem to indicate the relative tolerance of yard long bean to root oxygen deficiency itself. Thus, the conclusion obtained from the experiments with one variety in the former chapters could be confirmed in this chapter where the experiments with some number of varieties were conducted.

Chapter 5. General discussion

A series of experiments conducted here shows several aspects regarding responses of yard long bean to waterlogging. These aspects are discussed in relation to selection and breeding of yard long bean for waterlogging tolerance and cultural practices for preventing flooding damage.

As stated before, the adverse effects of waterlogging on plant growth result directly from restricted root aerobic growth caused by oxygen deficiency, and indirectly from the interaction of plant and environment, mainly soil environment. Agronomical, morphological and physiological data have proved that yard long bean is generally tolerant to root oxygen deficiency, although varietal differences in tolerance were clearly existent. Several studies, however, indicated that yard long bean and cowpea, which belongs to the same species as yard long bean, were susceptible to waterlogging (Minchin et al., 1978; Tomooka, 1982; Wien et al., 1979). Furthermore, under actual field conditions even very short-term waterlogging severely restricts the growth and yield of yard long bean and sometimes leads to the death of whole plant. These facts and the results obtained here appear to indicate that yard long bean is sensitive to some of indirect effects of soil waterlogging besides root oxygen deficiency.

Most of indirect effects of waterlogging are known to be mediated by soil microorganisms (Fitter and Hay, 1981). Various kinds of facultative and obligate anaerobic microorganisms are activated when soils are waterlogged. These anaerobic microorganisms maintain their respiratory activity using other electron acceptors than oxygen. Well known reactions mediated by those anaerobic microorganisms are as follows.

$$NO_3^- + 6\ H^+ + 5\ e^- \rightarrow 1/2N_2 + 3H_2O$$

$$MnO_2 + 4\ H^+ + 2\ e^- \rightarrow Mn^{2+} + 2H_2O$$

$$Fe(OH)_3 + 3\ H^+ + 1\ e^- \rightarrow Fe^{2+} + 3\ H_2O$$

$$SO_4^{2-} + 10\ H^+ + 8\ e^- \rightarrow H_2S + 4\ H_2O$$

$$CO_2 + 8\ H^+ + 8\ e^- \rightarrow CH_4 + 2\ H_2O$$

These reactions result in large loss of available nitrogen from rhizosphere by denitrification, accumulation of toxic inorganic ions such as Fe^{2+} and Mn^{2+} and production of gaseous compounds like H_2S or methane which influence plant growth and development adversely.

Additionally to those anaerobic microorganisms which induce many kinds of chemical reaction in soils, some of pathogenic microorganisms will be active under soil waterlogging conditions. Those microorganisms with pathogenic activities sometimes cause root rot or stem rot and give plants deleterious effects (McCalla and Haskins, 1964).

On the contrary to the activation of anaerobic microorganisms, activities of obligate aerobic microorganisms are severely retarded under waterlogging conditions. Among them the inactivation of root nodule bacteria gives detrimental effects on the growth of leguminous crops like yard long bean, which depend their necessary nitrogen on root nodule (Sprent, 1972; Minchin and Pate, 1975; Minchin and Summerfield, 1976; Jackson and Campbell, 1979).

Humid atmospheric conditions induced by soil inundation may also increase the activities of aerial pathogens. Although many reviews concerning plant response to waterlogging (Krizek, 1982; Bradford and Yang, 1981; Kawase, 1981; Fitter and Hay 1981; Glinski and Stepniewski, 1985) did not mention the invasion of aerial pathogens, this seems to be an important factor in actual crop production.

It is generally considered that those indirect effects of waterlogging are minimal for some duration after the onset of waterlogging and that become remarkable as the waterlogging duration prolongs (Jackson, 1985). In tropical environment, however, those indirect effects, especially several soil chemical reactions caused by anaerobic bacteria, may appear more rapidly than in temperate environment because of high air and soil temperature. Furthermore, as the respiration rates of both plant roots and soil microorganisms are much higher in tropical soils than in temperate soils, oxygen in the soil is more quickly depleted (Glinski and Stepniewski, 1985) and plant roots are exposed to oxygen deficiency more rapidly (Krizek, 1982). Thus combined, direct and indirect effects of waterlogging may appear even under short-term flooding in tropical environment (Wien et al., 1979). The result obtained in this study seems to support this view.

Considering the above-mentioned factors, plant responses to waterlogging can be expressed as follows;

$$R_w = R_d + R_i + I \quad --- \quad (1)$$

Here, R_w, R_d, R_i and I mean the responses to waterlogging, the responses to direct root oxygen deficiency, the responses to indirect effects of waterlogging and interactions between direct and indirect effects, respectively. The extent of the responses to direct effects is controlled mainly by plant genetic factors and influenced by environmental conditions. The responses include both damages caused by and adaptation to waterlogging. The extent of the responses to indirect effects is dependent on the characteristics of the environment, especially of soils. Indirect effects give plants both positive and negative influences. Thus tolerance of plants to waterlogging can be expressed as follows;

$$T_w = T_d + T_i + I \quad --- \quad (2)$$

Here, T_w, T_d, T_i and I correspond waterlogging tolerance, the tolerance to damages caused by direct effects, the tolerance to indirect, negative effects and interactions between them, respectively.

These aspects concerning the responses of yard long bean to waterlogging are true irrespective of the duration of the stress, but agronomically the responses to short-term waterlogging are more important, because under actual field conditions most crops experience only transient or short-term waterlogging. In this way, further research work must be conducted mainly to elucidate the plant responses to short-term waterlogging in order to proceed both selection and breeding for waterlogging tolerance and the development of cultural practices for minimizing waterlogging damages.

For efficient selection and breeding, it is required to evaluate the ability of plants to adapt to waterlogging conditions accurately. As shown in the equation (2), tolerant variety must have tolerance to both direct and indirect effects of the stress. This fact may give selection and breeding for waterlogging tolerance both inefficiency and difficulty. In the first step, T_d in equation (2) should be investigated under conditions where the influences of microorganisms are minimized. Then in the second step, T_i in equation (2) should be evaluated. Interaction between T_d and T_i must be studied in the final step. In leguminous crops, as the role of nitrogen fixing Rhizobia is especially important for their growth, the interaction between these bacteria and plant responses to waterlogging should be studied separately from that of the other microorganisms.

Selection and breeding for tolerance to direct root oxygen deficiency (T_d) seem much easier to conduct than those for tolerance to indirect effects of waterlogging (T_i), because the target trait is simple. Actually, plants should be grown in sterilized soils or in hydroponics in order to minimize the indirect effects mainly induced by soil microorganisms. This may restrict the large scale selections.

An additional problem for selection and breeding for tolerance to direct root oxygen deficiency is the existence of the dual stress caused by waterlogging. The first stress is caused by root oxygen deficiency and the second stress is caused by the removal of the stress itself after the partial or complete adaptation to the waterlogged environment. In this meaning, the tolerance to the first stress may be called 'adapting ability', and the tolerance to the second stress 'recovering ability'. As short-term waterlogging is more important agronomically, the research work must be done mainly on 'recovering ability'. Thus, criteria for the selection for the tolerance must be related to 'recovering ability' of plants. Among various morphological and physiological changes during or after the waterlogging period, the formation of white and spongy tissue and the development of adventitious roots seem to have close relationship with 'adapting ability' of plants. These characteristics are ecologically important when we consider the adaptive feature of yard long bean plant to root anaerobiosis, but their importance is low agronomically. On the contrary, the maintenance of aerobic respiratory apparatus in primary roots is supposed to be closely associated with 'recovering ability' of plants and may have relatively higher importance.

Selection and breeding for the tolerance to indirect effects of waterlogging is considered to be very difficult to achieve, because various constraint is included in the plant responses to the indirect effects as described before. It appears nearly impossible to breed out a variety which has the tolerance to all the damages induced by the indirect effects of waterlogging. At the present level of knowledge, it is better to counteract the indirect effects of waterlogging by the development of cultural practices.

Until recently more emphasis has been placed in crop improvement by selection and breeding, in comparison to cultural practices for better plant management, from the standpoint of the increasing economic limits imposed on tropical small farmers. But the advancement of productivity under the stress conditions requires both the improvement of plant material and the development of agronomical practices. These two factors are equally important.

It is quite natural that a cultural practice such as large-scaled construction for the modification of local topography or for the improvement of drainage system is effective to diminish the damages caused by soil waterlogging, but inexpensive practices which can be adopted by individual tropical farmer are also possible and effective. The results obtained in these experiments may give useful suggestions on such cultural practices to minimize waterlogging damage. For instance, adjustment of the planting date is beneficial for minimizing the damage, if a given variety shows a sensitive period and if the occurrence of heavy shower is anticipated. As the yield reduction caused by short-term waterlogging results mainly from the restriction of lateral shoot development, the increase of the plant density may be useful for stabilizing the yield. Various kinds of soil sterilization are also considered to be effective.

In some regions of tropical Asia, production systems have been developed to adapt plants to environments in which waterlogging is expected to occur frequently. Vegetable producing areas near Bangkok in Thailand (Nawata, 1986) and floating islands in the Inlay Lake in Burma (Hsan, 1988) are representative examples. Inundation of whole field before planting in the former case, and burning of field before planting in the latter case, appear to enable vegetables to grow without sustaining flooding damage. Both methods seem to sterilize the soils effectively. Detailed survey on plant management in these areas should supply more information about the development of cultural practices for minimizing waterlogging damage.

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